

## Initial Infection of *Falcataria moluccana* Leaves and *Acacia mangium* Phyllodes by *Uromycladium tepperianum* Fungi in a Laboratory Trial

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### Abstract

*Sengon* is a fast growing species that is cultivated widely in Indonesia. Lately, *sengon* is severely attacked by fungus *Uromycladium tepperianum* that causing gall rust disease. It is also known to attack various types of acacia. This study aims to determine the fungal infection process *U. tepperianum* on *sengon* leaves and the possibility of infection on *Acacia mangium* in the laboratory trial. Leave samples and fungal pathogen teliospores obtained from Cangkringan, Sleman, Yogyakarta. Several approach procedures conducted to achieve these objectives were: (1) identification of diseased trees, (2) collecting samples of diseased leaves, branches, twigs, and stems, and (3) artificial inoculation and investigating the infection process of *U. tepperianum* teliospores in the laboratory. The results showed that the process of infection in *sengon* started by teliospores germination and germ tube formation. Successive germ tube forming penetration pegs. In the plant tissue, the penetration peg formed hypha and further developed into intracellular and intercellular hyphae. The artificial inoculation on *A. mangium* leaf surface showed few spores can germinate. However, none of them managed to penetrate.

Keywords: *Uromycladium tepperianum*, *sengon*, acacia, gall rust, infection

### Abstrak

*Sengon* merupakan tanaman cepat tumbuh yang dibudidayakan di Indonesia. Namun, akhir-akhir ini banyak *sengon* yang menderita penyakit karat tumor sangat parah. Penyakit ini disebabkan oleh jamur *Uromycladium tepperianum*, juga diketahui menyerang berbagai jenis tanaman akasia. Penelitian ini bertujuan mengetahui proses infeksi jamur *U. tepperianum* pada daun *sengon* dan kemungkinan infeksinya pada *Acacia mangium* di laboratorium. Sampel dan teliospora jamur patogen didapatkan dari Cangkringan, Sleman, Yogyakarta. Beberapa pendekatan yang digunakan untuk mencapai tujuan tersebut adalah: (1) identifikasi pohon yang sakit, (2) pengumpulan contoh berupa daun, cabang, ranting, dan batang yang sakit, serta (3) inokulasi buatan dan proses infeksi *U. tepperianum* di laboratorium menggunakan teliospora *U. tepperianum*. Hasil penelitian menunjukkan bahwa proses infeksi pada tanaman *sengon* diawali dengan perkecambahan teliospora dan pembentukan buluh kecambah. Buluh kecambah selanjutnya membentuk pasak penetrasi. Di dalam jaringan tanaman, pasak penetrasi membentuk hifa dan berkembang menjadi hifa intraseluler dan interseuler. Hasil uji inokulasi buatan pada permukaan daun *A. mangium* menunjukkan sebagian kecil spora dapat berkecambah. Namun, tidak ada yang berhasil melakukan penetrasi.

Kata kunci: *Uromycladium tepperianum*, *sengon*, akasia, karat tumor, infeksi

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## Introduction

*Sengon* (*Falcataria moluccana* (Miq.) Barneby & JWGrimes) is a well-known tree in the smallholder plantation, particularly in Java. It is a fast growing tree that has diverse use of its timber. It is provide raw material for several industries such as pulp and paper production, plywood, fiber-board, and block-board. Given the high economic value and the ease of management, the cultivation of *sengon* is increased (Atmosuseno 1998). As a fast growing tree it can height up to 45 m with diameter up to 100 cm over 25 years. At the age of 6 years *sengon* plantations can produce

as much as 372 m<sup>3</sup> of logs ha<sup>-1</sup>, so it is described as a miracle tree (Atmosuseno 1998).

Natural distributions of *sengon* are ranging from Maluku, Papua New Guinea, Bismark Islands, to the Solomon Islands. However, currently *sengon* widely planted in tropical climate area (Soerianegara & Lemmens 1994). *Sengon* does not require complicated requirements to grow. *Sengon* has ability to grow on a variety of soil types, ranging from poor to well drained soil, and from marginal land to soil that rich of nutrients. In Brunei and Malaysia, *sengon* can grow in saline, dry, and moist soils. It grows well in the altitude

ranging 0–1,500 m asl. However, optimal growth of *sengon* obtained when planted in nutrients rich soil with good texture and structure (Atmosuseno 1998). Best soil for *sengon* is the soil with pH 6–7. *Sengon* is a tropical tree species that will grow well at a temperature of 18–27 °C with a humidity of 50–75%. *Sengon* prefers to expose to sufficient amount of sunlight, therefore it should be planted in the open land (Santoso 2000).

In the addition to *sengon*, *Acacia mangium* was also used in this study. It is a fast growing tree that harvested between 8 to 10 years old (Joker 2001). Natural distribution of *A. mangium* includes Sulawesi, Seram, Aru Island, Papua, Papua New Guinea, and northeastern Queensland (Lemmens & Soerianegara 1995). Distribution of these plants is strongly influenced by rainfall and soil drainage. *A. mangium* optimally grows on an altitude of 100–780 m asl. It is a pioneer species in lowland rain forest, critical land and dry land with a low pH (4.5–6.5). It is also grow in the lands close to mangroves, in the seasonal swamps, along rivers side, dry plains, hills, and in the mountain-foot (Awang & Taylor 1993).

*A. mangium* can reach up to 30 m height with the clear boles can be more than half of its height. It has rough surface, grooved longitudinally, and the color varies from pale grey brown to brown. Buttress roots are sometimes found at the base of the trunk (Turnbull 1986). Although there are no reports of *U. tepperianum* attacks on *A. mangium* to date, but as an invasive species, its stands are susceptible to various diseases.

Currently *sengon* has gain wide interest among small timber companies and smallholder farmers to cultivate it. To our knowledge, an extensive cultivation of limited species which tend to single type cultivation (monoculture) is prone to the development of plant pests and diseases. It has a great possibility to develop into epidemic (Wiryadiputra 2007).

One of the problems that have arisen with the extensive cultivation of *sengon* at the moment is the outbreaks of gall rust disease that caused by a fungus *Uromycladium tepperianum*. It can cause the death of *sengon* from seedlings to the stands (Rahayu *et al.* 2009; Anggraeni *et al.* 2010; Rahayu *et al.* 2011). The gall rust disease has also been reported caused huge losses to *sengon* plantation owned by government, private, and smallholders in Philippines (Braza 1997), East Timor (Old 2003), and Malaysia (Lee 2004). The fungi also attacks *sengon* which provide shade for coffee plantations in East Timor and causing damage to coffee plants as the shade trees are damaged or even dead (Old 2003).

The gall rust disease rapidly disperses by the wind and no proper and effective way to control it to date. Several studies abroad indicate that the fungus *U. tepperianum* can also infect various types of acacia (Old *et al.* 2000). *Acacia mangium* is a species that grow widely in Indonesia. Both private and state plantation industry in Indonesian have planted several types of acacia as a component of industrial plantation forest (HTI) including *A. mangium*. It is feared that this species may also be infected by the *U. tepperianum*. This research was conducted to determine the initial infection of *U. tepperianum* *sengon* leaf and the possibility of fungal infections of *U. tepperianum* on *A. mangium* phyllodes.

## Methods

Preliminary observation of *U. tepperianum* attacks in *sengon* was performed in *sengon* community plantation forest (*hutan rakyat*) in Wonosobo, Central Java, while the sample of diseased stems, branches, twigs, and leaves were collected from *sengon* community plantation forest in Cangkringan, Sleman, Yogyakarta. The further procedures of study were performed in the Laboratory of Forest Protection and Health, Faculty of Forestry, Gadjah Mada University.

**Diseased tree identification** Identification was conducted by observing and recording the trees in the field that show symptoms and signs of disease. Symptom of enlargement of size was observed on the stem, branches, twigs, and leaves. The formation of dark brown spores mass (powdery spores) was also observed.

**Sample collection** Sampling of diseased trees in the field was conducted by cutting the leaves, branches, twigs, and stems which indicate the presence of a gall using a scissors or a hand saw. The samples were then kept in a paper envelope and marked with a permanent marker. Samples were protected from wilting by avoiding hot condition and immediately taken to the laboratory for further observation. Leaf and phyllode were selected as a material for inoculation as it is easy to collect and to observe. However, the leaf sample was provides only single observation opportunity since it can not be observed furthermore if the tissues had died.

**The infection process of *U. tepperianum*** *U. tepperianum* produced a single type of spores called teliospores (Rahayu 2008). Teliospores found on the surface of the gall were removed by a small brush and placed in a petridish. The teliospores were dissolved in sterile water that contains Tween-20 (0.02%). Tween-20 will saturate binding sites on water surfaces and helps teliospores stick to the surface of plant organs. Teliospores concentration was  $1 \times 10^5$  spores  $\text{mL}^{-1}$ . Teliospores suspensions were then inoculated on leaves of *sengon* seedling and phyllodes of *A. mangium* using a soft brush. Inoculated seedlings were placed in a container to maintain the humidity and temperature was kept between of 25–30 °C for 96 hours (modified Morris 1987). The treatment was applied to 4 months old seedlings of *sengon* and *A. mangium*, each treatment contains 6 seedlings.

Observations and image is taken at 2, 24, 48, 72, and 96 hours after teliospores inoculation. Twenty leaves of *sengon* and 5 phyllodes of *A. mangium* that had been inoculated with teliospores solution were taken for each observation. For *A. mangium*, phyllodes were cut into 1  $\text{cm}^2$  for ease treatment and observation. Samples were placed in a 50 mL Erlenmeyer flask that contains 20 mL of 96% ethanol. Samples in ethanol solution were then boiled for 20 minutes to remove the chlorophyll (Ruzin 1999), then 96% ethanol was removed and replaced with chloral hydrate ( $2.5 \text{ g mL}^{-1}$ ), followed by another heating for 20 minutes until the sample becomes transparent (Elliott *et al.* 2008). To obtain a cross-sectional sample, the leaf was longitudinally cut using a microtome.

A model of infection process of *U. tepperianum* to plant species can be studied using some selected leaf for the artificial inoculation. In this study, *A. mangium* was selected in addition to *sengon*. The morphology of thin leaves provides better of observation, through the upper surface of the leaves as well as on the cross section. The relatively large number of leaves on *sengon* and *A. mangium* also provides enough samples for observation and reduces the number of seedling required to perform the experiment. The multiple layers of leaf tissue and the presence of stomata on the leaf surface provide a clear image of the infection process occurred.

Observation of inoculated samples was conducted using Olympus CX31 microscope. Leaf sample was placed on glass objects and stained with laptophenol trypan blue using a pipette. The image of microscope observation was recorded using a MDCE-5A digital camera that connected to a computer that installed with ScopePhoto version 2.0.4 software.

The number of teliospores that germinate and penetrate to 5 leaves of *sengon* and 5 phyllodes of *A. mangium* was counted to determine the tendency (trend) of germination and penetration in the leaves of 2 species used in this study. The observed teliospores were grouped into 3 categories, i.e.; non germinated teliospores, germinated teliospores, and penetrated teliospores.

## Results and Discussion

**Symptoms and signs of gall rust disease of *sengon* in the field** *Sengon* is a susceptible plant to attack by pathogens, such as fungi *Ganoderma* sp. (Herliana *et al.* 2012), *Oidium* sp., and *U. tepperianum*. This tree is highly cultivated by community and government companies in the sampling location in Cangkringan. *Sengon* stands in this site are showing symptoms and signs of disease in nearly all of the trees (Figure 1).

Gall rust disease found in *sengon* stands is identified caused by *U. tepperianum*. It is a rust fungi that causes gall forming and attacks several types of *Acacia* spp. *U. tepperianum* is a native Australian fungus that was introduced to South Africa as a biological control agent in 1987 (Wood 2012). It was used to control *Acacia saligna* in South Africa, with no expectation to attack other plant species (Morris 1997; Mehta 2000). However, it was contrary to the results of study conducted in Philippines that *U. tepperianum* was attacked *sengon* (Braza 1997). It was also causing outbreaks in *sengon* plantation in Sabah, Malaysia (Lee 2004) and attacked *sengon* that serves as shade tree in coffee plantations in East Timor (Old 2003). The symptoms of this disease in *sengon* notice by a gall that locally found in the infected trunk, branches, twigs, leaves, and stalks. *U. tepperianum* has small pycnium, blackish brown color, globose with a diameter of 150  $\mu\text{m}$ , spermatia hyaline, and ellipsoid shape. Galls grow from less than 1 cm in diameter to about 10 cm, the swelling of the trunk and branches can even greater than 18  $\times$  6 cm with a reddish brown color to dark brown. Gall shape varies from round to irregular. The young galls are green (Figure 2b) and develop further until it all covered up with a layer of reddish brown rust powder (Figure 2e). The powdery layer is a deposit of

teliospores, which is a sign of this disease. Teliospores composed of a cluster of three probasidial cells that are located at top of a single pedicel, depressed between globoses, with reddish light brown color, vertical thick striate, crenulate margin. The thickness of spore wall is 2–3  $\mu\text{m}$ , with up to 5  $\mu\text{m}$  thickness in the apex. The length of teliospores is between 14–22  $\mu\text{m}$  and 18–25  $\mu\text{m}$  width. It has one apical germ pore, pedicel hyaline, septate, and deciduous (USDA 2007). If the disease hit the petiole, it will cause petiole to bend due to the thickening and swelling, the leaf will curl and eventually shed.

A leaf sample infected by *U. tepperianum* shows symptoms of disease with discoloration and changes in shape by curling in one side of leaf margin because of swelling (Figure 2a). This is consistent with Lee's study (2004) that discoloration and deformation are the symptoms of gall rust diseases. Figure 2b shows that infection occurs in the petiole, which is analogous to a research by Wiryadiputra (2007), the infected petiole was swollen and bent irregularly, it will ended up with yellowing and casting of the leaves. Swelling and curling was also occurs in the infected twigs (Figure 2c). Figure 2d and 2e show the swelling that occurs in the main stem of the plant. The diameter of gall in the branch and trunk can be > 5 cm. Usually in the larger gall teliospores are more abundance and noticed by reddish brown powder (Wiryadiputra 2007).

In the most of the pathogenic fungi, spores are their propagules that widely spread by several factors such as wind, insects, birds, and humans (Agrios 2005; Triyogo & Widyastuti 2012). Spores of *U. tepperianum* disperse widely as the wind will easily blow them out. Insects also serve as vectors of gall rust disease. In a study conducted by Triyogo & Widyastuti (2012) it was revealed that 5 orders of insects (*Hemiptera*, *Diptera*, *Hymenoptera*, *Coleoptera*, and *Lepidoptera*) were associated with galls in *sengon*. This study also recognized that lepidoptera helps the spread of *U. tepperianum* spores. Another study has discovered that the old galls usually have holes as it inhabited by insects and turn black as they were experienced decay (Anggraeni & Santoso 2003).

**Early infection of *U. tepperianum* on *sengon* leaves in the laboratory** Infection is one of the important things in the disease process. Infection in older plants generally resulted in alteration of plants form and may reduce its vigor, while in seedlings and young plants it may resulting death (Edmonds *et al.* 2000; Rahayu *et al.* 2010; Triyogo & Widyastuti 2012). According to Siddiqui *et al.* (2009), the ability of a pathogen to infect mature plants was lower than in young plants. Previous studies stated that severity of infection declines with decreasing density of the host (Wood 2012). *U. tepperianum* is a rust fungus that produces single microcyclic spores. The mycelium forms pycnia that finally produces teliospores (Wiryadiputra 2007). An obligate fungus such as rusts can not grow on artificial media (Perfect & Green 2001). Therefore, teliospores for pathogenicity test were collected directly from *sengon* stems infected by *U. tepperianum* (Figure 3). To observe the process of the initial infection of *U. tepperianum* leaves were selected as materials for artificial inoculation.

Observations at 2, 24, 48, 72, and 96 hours after inoculation found that teliospores inoculated on sengon leaf surface were able to germinate and penetrate. Figure 4 shows the development of teliospores germination and penetration, after two hours of inoculation teliospores on sengon leaf surface do not show any progressions (Figure 4).

Observation of the samples after 48 hours of inoculation (Figure 4b) shows that teliospores on the leaves surface were germinated and formed a germ tube that extends to the surface of the leaf, Germ tube is then developed into penetration pegs. According to Agrios (2005), penetration pegs generally have a smaller diameter than normal hyphae. If the penetration peg successfully entered into the plant tissue, it will return to the same diameter as normal hyphae. The penetration pegs which penetrate directly on the epidermal cells wall of leaf surface will form vesicles in the epidermal cells. The result is analogous to research by Morris (1987) that used teliospores collected from *A. saligna* organs infected by gall rust disease as inoculums and inoculated them on various species of acacia.

In further developments, the vesicles were formed hyphae in epidermis cells (Figure 4c). The hyphae penetrate into inner epidermis cell wall and then form the intercellular hyphae (Figure 4d). According to Morris (1987), these intracellular hyphae then headed to transporter tissue through the mesophyll tissue. In natural infections, teliospores formed basidiospores at the beginning then form penetration pegs and finally penetrates the plant tissue. During the natural infection process in sengon, *U. tepperianum* teliospores on the leaf surface were germinated to form basidiospores. In favorable conditions i.e. high relative humidity ( $\geq 90\%$ ), basidiospores were formed at 10 hours after inoculation and 6 hours after the penetration pegs were formed and directly penetrate the cells in the epidermal layer of the host (Rahayu *et al.* 2010). The differences of infection process may be caused by the differences in the environmental conditions.

**The development of *U. tepperianum* teliospores on sengon leaves and *A. mangium* phyllodes** The observation of the development of teliospores of *U. tepperianum* on the sengon leaf surface and *A. mangium* phyllodia performed in the laboratory are presented in Figure 5. In the observation of sengon leaves at 24 hours after inoculation, 145 out of 640 teliospores were found germinated, and only 87 spores were penetrated. While the observation at 48 hours after inoculation found 182 out of 721 teliospores germinated and 103 teliospores were penetrated.

In the observation at 72 hours after inoculation, 195 out of 826 teliospores were germinated and only 128 spores were penetrated. In this study the development of teliospores on sengon leaves tends to increase by hours of inoculation, both the number of germinated and penetrated teliospores, although there were some teliospores still unable to germinate. According Widyastuti *et al.* (2005), the failure of teliospores to germinate during inoculation generally caused by the presence of inhibitory compounds that formed during the process of sporulation. Observations on the fourth day were unable to be undertaken due to the abundance of germ tubes and penetration pegs that grow on the leaves surface.

They were causing obstruction to the observations and causing no accurate data will be obtained.

In contrast to the results of teliospores observed on sengon leaves, teliospores in *A. mangium* phyllodes observed at 24 hours after inoculation found out a total of 1,954 teliospores in the phyllodes surface, but no teliospores were germinated and penetrated. In the observation at 48 hours after inoculation found 81 out of 4,276 teliospores germinate, but none were able to penetrate. In the observation at 72 hours after inoculation found 110 out of 2,961 teliospores were germinated and again none of the teliospores were penetrated. In the samples observed 96 hours after inoculation found 181 out of 4,309 teliospores were germinated and none of the teliospores were penetrated. The results indicated that the number of teliospores germinated in *A. mangium* phyllodes also tends to increase with time, while the penetration was never occur. Previous studies conducted by Morris (1987) on *A. mangium* using teliospores collected from of *Acacia implexa* as inoculum, indicates that there was a resistant reaction causing the growth of hyphae were stopped. Germinating teliospores were surrounded by necrosis and chlorosis, and no galls were formed.

Figure 6 shows the failure of infection of teliospores in *A. mangium* phyllodium. The teliospore in *A. mangium* phyllodium surface was germinated and formed a germ tube (Figure 6a). The germ tube invades the tissue surface and develops penetration pegs. However the epidermis of *A. mangium* phyllodium has a thick layer of wax (Awang & Taylor 1993), which provides an initial defense and prevent pathogens to penetrate the surface of the phyllodium. The wax coating provides protection to plants from pathogen penetration.

Penetration pegs were formed on the surface of phyllodes of *A. mangium* (Figure 6b). The formation of penetration peg generates a signal that can be recognized by *A. mangium* to activate the natural defense response of plant. Yellowish brown color at the penetration site suggests a defense reaction of *A. mangium*. The defense alleged to be the accumulation of phenolic compounds, according Lattanzio *et al.* (2006) which states that if the outer defenses of the plant is unable to stop the infection process of the pathogen, the plant will respond by increasing phenolic content at the site of infection to create unfavourable conditions for further infection process. The series of self-defense shown by *A. mangium* is a non-host resistance. *U. tepperianum* only need one single host to finish whole life cycle which is sengon (Rahayu 2008). In addition to the infection process that does not occur in *A. mangium*, this study suggests the small possibility of *U. tepperianum* attacking *A. mangium*.

## Conclusion

Initial infection of *U. tepperianum* as a result of artificial inoculation on sengon leaves in a laboratory trial begins with direct penetration of penetration pegs to epidermal cells of host plant. Once infection occurs, the penetration peg produces vesicles and hypha inside plant tissue. An artificial inoculation of teliospores on *A. mangium* phyllodes showed that infection does not occur.



Figure 1 *Sengon* infected by *Uromycladium tepperianum* in the community forest stand. Red arrows show the gall rust in the branches.



Figure 2 Symptoms of gall rust diseases at sengon organs: (a) leaves, (b) leaf stalks, (c) branches, (d) and (e) trunk.

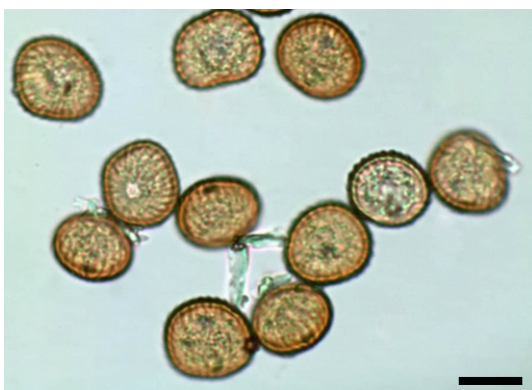


Figure 3 Teliospores of *Uromycladium tepperianum*, collected from trunk surface of sengon showing gall rust. These teliospores were used as inoculum source for artificial inoculation study on sengon leaves.

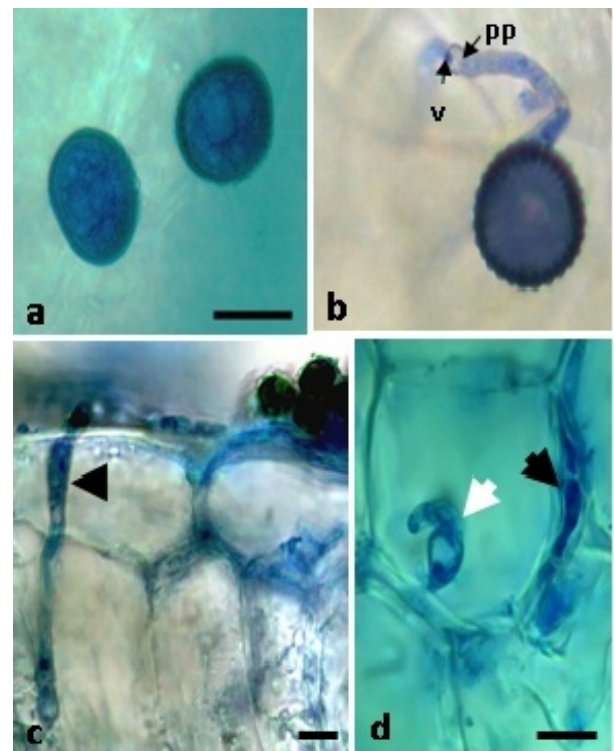


Figure 4 Infection process of *Uromycladium tepperianum* on *sengon* leaves by artificial inoculation. (a) Teliospores on leaf surface (2 hours after inoculation), (b) germinated teliospore (24 hours after inoculation) pp: *penetration peg*, v: *vesicle*. (c) Direct penetration of intracellular hyphae (black arrow) (48 hours after inoculation), (d) intracellular hyphae (white arrow) dan intercellular hyphae (black arrow) on spongy mesophyll (96 hours after inoculation). Figure (a) and (b) are observed on leaf surface, (c) and (d) are observed from longitudinal cross section of leaf. All samples were stained with *lactophenol trypan-blue*. Bars are representing 10  $\mu$ m lengths.

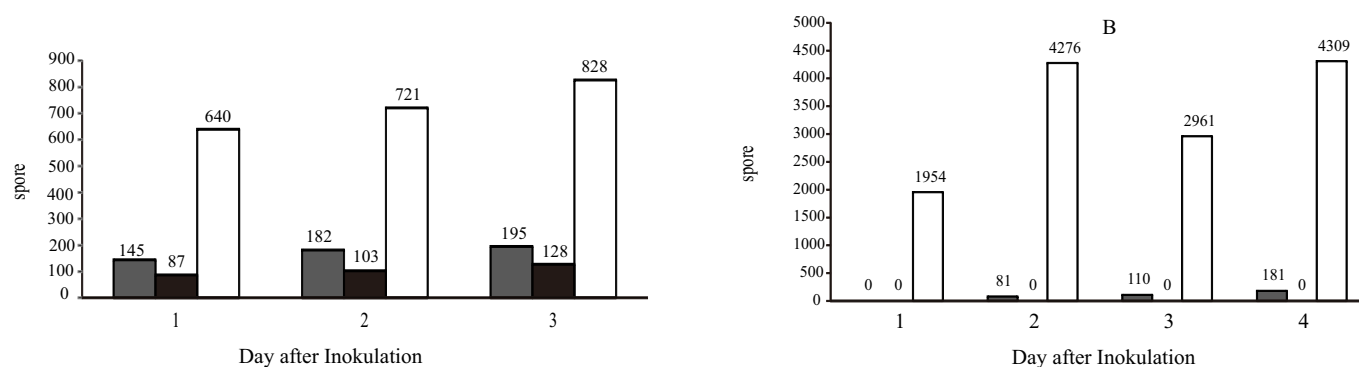


Figure 5 The number of germinated and penetrated teliospores on: (a) *sengon* leaves, (b) *Acacia mangium* phyllodium. Total germinated (■), total penetrated (■), total teliospora (□)

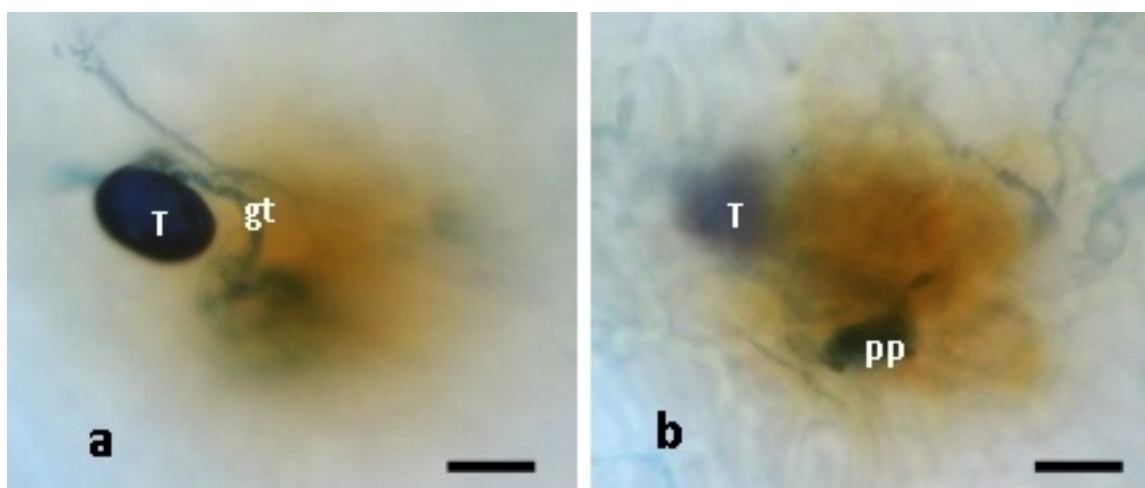


Figure 6 The final of infection process of *Uromycladium tepperianum* on surface of *Acacia mangium* phyllodium 2 days after inoculation, (a) and (b) are the same object observed by different focus under microscope. T: teliospore, gt: germ tube, pp: penetration peg. Brown color surrounding infection site are assumed as phenolic compound deposit. Sample is stained with *lactophenol trypan-blue*. Bars are representing 10  $\mu$ m lengths.

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