EFFECTS OF TAR SMEARING TREATMENT ON *FALCATARIA MOLUCCANA* INFECTED BY GALL RUST DISEASE

H Cipta, S Rahayu & WD Nugroho*

Faculty of Forestry, Universitas Gadjah Mada, Jalan Agro No. 1 Bulaksumur, Yogyakarta, 55281, Indonesia

**wdnugroho@ugm.ac.id*

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Gall rust disease caused by *Uromycladium tepperianum* fungus is one of the destructive diseases of *Falcataria moluccana* in Indonesia. The fungus induces gall formation either on stem, branch or trunk of young and mature trees. Control practice using tar solution for preventing gall reformation on stem, after removal of gall, has been conducted. However the effects of tar in inhibiting gall reformation, especially on the anatomical properties of *F. moluccana* xylem, are not known. This study aimed to find out the effects of tar treatment on gall reformation and the anatomical properties of *F. moluccana* xylem after treatment. Three kinds of treatments were applied on the trunk after the gall was removed, namey, one time tar smearing, per 15 days tar smearing and without tar smearing. Observed parameters were number of gall reformation and wood anatomical properties modification. The result indicated that tar treatments reduced gall reformation. Significant modifications on wood anatomical properties occurred in one time tar smearing treatment i.e. wood fibre diameter and ray width. The effects of gall rust on *F. moluccana* wood were observable through appearance of fungi mycelia in fibre cell, formation of multiseriate ray cells, presence of phenolic-like compound and modification on axial parenchyma cells configuration and narrower vessel element.

Keywords: Coppice, fungal disease, wood anatomy, community forest, *Uromycladium tepperianum*

INTRODUCTION

Falcataria moluccana is a fast growing multipurpose tree species, widely planted in Indonesia. The height of *F. moluccana* can reach up to 7 m in 1 year, 15 m in 3 years and 39 m in 10 years, with an average wood volume yield of 39 $m^3 ha^{-1} year^{-1}$ in a 10-year rotation, and may reach 50 $m^3 ha^{-1} year^{-1}$ on better soil (CABI 2014). *Falcataria moluccana* is also known as a native plant in Haiti, Indonesia, Papua New Guinea and Bismarck Archipelago (Hanum & van der Maesen 1997, Hartati 2008). In Java, Indonesia, *F. moluccana* is broadly developed in community forest because of its various advantages (Nemoto 2002, Sumedi 2008, Indresputra et al. 2013). *Falcataria moluccana* belongs to the family Leguminosae and subfamily Mimosoideae. *Falcataria moluccana* has synonims, such as *Albizia moluccana, A. falcata, A. falcataria* and *Paraserianthes falcataria* (ACTI 1983). *Falcataria moluccana* is a valuable species with multiple uses. Its wood is used for pulp, light construction, furniture, cabinet works, lightweight packing material, pallets, match wood, wooden shoes, musical instrument, toys and novelties, form and general turnery, lightweight veneer and plywood, particleboard, wood-wool board hardboard, blockboard, and fire-resistant doors (Hanum & van der Maesen 1997, Nemoto 2002).

Gall rust disease, caused by *Uromycladium tepperianum* (Old & Cristovao 2003, CABI 2014), is one of the most dangerous diseases of *F. moluccana* in South-East Asia including Indonesia (Hanum & van der Maesen 1997, Rahayu et al. 2010). *Uromycladium tepperianum* could also attack other tree species. Morris (1987) reported that more than 100 *Acacia* species could be attacked by *U. tepperianum*. The response of each plant towards *U. tepperianum* is different, either on different host or same host in different habitats (Burges 1934).

This disease is responsible for the financial loss of *F. moluccana* plantations. Replacement of species can be an option to counteract the disease, however it involves a big cost (Old & Christovao 2003, Rahayu et al. 2010, Wood 2012). On the other hand, due to its destructive

characteristic, the disease was used as a biological control against the invasive species, *Acacia saligna*, in South Africa (Morris 1987, Morris 1999, Wood & Morris 2007).

Uromycladium tepperianum infection on *F. moluccana* can occur on seed, seedling, young plant and mature plant as well as all parts of plant (Rahayu et al. 2010, Rahayu 2014). On mature *F. moluccana*, infection of trunk tissue by *U. tepperianum* causes hyperplasia by formation of subglobular-shaped gall on the surface of the trunk. The detailed gall anatomy has been explained (Burges 1934). Gall rust attack on the main trunk inhibits growth of tree, thus easily broken by strong wind (Rahayu 2014). Fungal attack on plant with various symptoms on trunk surface such as gall cancer or without gall or cancer symptom causes a modification on xylem cells (Peterson & Shurtleff 1965, Sakamoto et al. 2004, Angeles et al. 2006, Schweingruber 2007). In this case, gall rust caused by *U. tepperianum* significantly modified *F. moluccana* xylem. Several modifications on xylem anatomy of *F. moluccana* consist of narrower size and lower percentage of vessel element, increased percentage of parenchyma (axial and ray parenchyma) cells, as well as modification of axial parenchyma type (Rukhama & Nugroho 2014).

Similar response occured on other rust fungi attack. The xylem anatomy of *Pinus takahasii* attacked by gall rust-induced fungus, *Cronartium quercuum,* was modified, shown by short and twisted tracheid, increased height of rays and increased resin duct (Liping et al. 1990). The change in xylem anatomy caused by rust fungi (*Endrocronartium harknesii)* was also observed on *Pinus cortanta* (Zalasky 1976). The anatomy changes of xylem also occur on species attacked by other agents, such as bacteria. The attack of *Agrobacterium tumafaciens* that induced crown gall on *Lycopersicon esculentum* and *Ricinus commmunis* also modified xylem arrangement (Aloni et al 1995, Aloni et al 1998). Due to damage caused by gall rust on *F. moluccana* trunk, the attempt to control gall reformation, through treatment of removing gall followed by tar smearing, was conducted regularly, every two weeks. This treatment was able to prevent gall reformation on the trunk (Sari 2013). However, the treatment cannot stop the development of *U. tepperianum* in xylem located beneath the smeared area, especially in trees originated from coppice (Rahayu 2014).

This study aimed to find out the effects of tar treatment on gall reformation and the anatomical properties of *F. moluccana* xylem cells located beneath the treated area. The results of this study provided useful information about gall rust disease on *F. moluccana*.

MATERIALS AND METHODS

Materials

The study was conducted in a community forest located in Balong Village, Sleman Regency, Yogyakarta Province, Indonesia (110° 26' E and 7° 37' S) on altitude 734 meters above sea level, where five years old *F. moluccana* coppice trees are standing. Most of the coppice trees in this area were infected by gall rust disease indicated by gall rust symptom on the plant parts. Nine infected coppice trees with vertical elongated gall rust symptom on lower trunk were randomly chosen. The trees had a diameter of 8–15 cm. The symptom was shown by the presence of subglobose gall on the trunk as shown in Figure 1. The material used to prevent gall reformation was tar solution from PT Bintanglima Anekawarna, an Indonesian paint manufacturer.

Figure 1 *Falcataria moluccana,* attacked by gall rust disease, showed by the presence of subglobose gall on tree trunk

Tar smearing treatments

The gall developed on *F. moluccana* trunk was removed using a knife. Three kinds of treatment i.e. one time tar smearing (TA), per 15 days tar smearing (TB) and without tar smearing (TC) were applied on the removed area of *F. moluccana* trunk. Tar smearing was carried out by brushing tar solution on entire removed area ± 1 cm outside the border. Each treatment was applied on three *F. moluccana* trees. Observation of gall reformation on the stem was carried out regularly as mentioned in Table 1. Sample collection was conducted by removing a small wood block from each tree at the outermost part of the removed area, to observe wood anatomy properties. These blocks were removed randomly, using scalpel and chisel, and fixed in 4% glutaraldehyde solution. After 24 hours, the blocks were washed with and soaked in 30% ethanol.

Observation and measurement of characteristics of wood anatomy

The 1 cm × 1 cm × 1 cm block was prepared from the block obtained during sample collection. Transverse and tangential sections of 15 µm were cut from the block on a sliding microtome. The sections obtained were stained with 1% safranin and dehydrated in xylol, mounted on glass slides and fixed in Canada balsam. Images were taken with a light microscope equipped with camera. From the digital images, several characteristics of wood anatomy were observed and measured i.e. proportion, morphology and structure of wood cell types. Proportion and morphology of wood cell types were observed using image analysis software. Proportions of cell types measured were percentage of fibre, axial parenchyma, ray parenchyma and vessel element cells. Proportion of each cell types (%) was ratio of total area of each cell type per total area of measured image on transverse section (Nugroho et al. 2012). Morphology of cell types consisted of diameter of vessel element and fibre diameter, as well as height and width of ray parenchyma cells.

Measurement of vessel lumen diameter and fibre diameter was conducted using transverse sections. Vessel lumen diameter was determined by measuring tangential diameter of vessel lumen, excluding the wall, at the widest part of the opening. Fiber diameter was determined by measuring radial diameter of fibre cell. Measurement of height and width of ray parenchyma was conducted using tangential sections. Height of ray parenchyma was determined by measuring the vertical distance from tip to tip as seen in the tangential section. Width of ray parenchyma was determined by measuring the widest part of the ray parenchyma cells, perpendicular to the ray axis.

Statistical analysis

PASW Statistics 18 was used to analyse quantitative data. The relationship between time of treatment and characteristic of wood anatomy, including proportion and morphology of wood cell type parameter, was analysed using a one-way analysis of variance, followed by Tukey's post hoc test.

RESULTS AND DISCUSSION

Gall reformation

As shown in Table 2 and Figure 2, TB during 41 days reduced gall reformation, shown by the absence of gall on the surface. By contrast in TA and TC, the gall reformation was seen on day 21 and day 9 respectively. Similarly, TB on *F. moluccana* trunk was able to reduce gall reformation (Sari 2013). Gall reformation on the edges showed that gall could only be formed upon living tissues of *F. moluccana* (Figure 2). The study showed that *U. tepperianum*, similar with other rust fungi (order Pucciniales, previously also known as Uredinales), is an obligate parasite that can only complete its life cycle by exploiting suitable living host (Brian 1967).

Table 1 Observation and sample collection schedule

Year	Month	Date (Day)
2013	July	7 (day 0), 10 (day 3), 13 (day 6), 16 (day 9), 19 (day 12), 22 (day 15), 28 (day 21)
	August	3 (day 27), 9 (day 34), 16 (day 41)

Table 2 Observation of gall reformation

Treatments	Tree code	Day									
		0	3	6	9	12	15	21	27	34	41
One time tar smearing	TA1	0	0	0	0	0	0	+	+	+	+
	TA2	0	0	0	0	0	0	0	0	0	0
	TA3	0	0	0	0	0	0	0	0	0	0
Per 15 days tar smearing	TB1	0	0	0	0	0	0	0	0	0	0
	TB1	0	0	0	0	0	0	0	0	0	0
	TB3	0	0	0	0	0	0	0	0	0	0
Without tar smearing	TC1	0	0	0	0	0	+	+	+	+	+
	TC2	0	0	0	0	0	0	+	+	+	+
	TC3	0	0	0	+	+	+	+	+	+	+

0 = no gall reformation, + = gall reformation occured

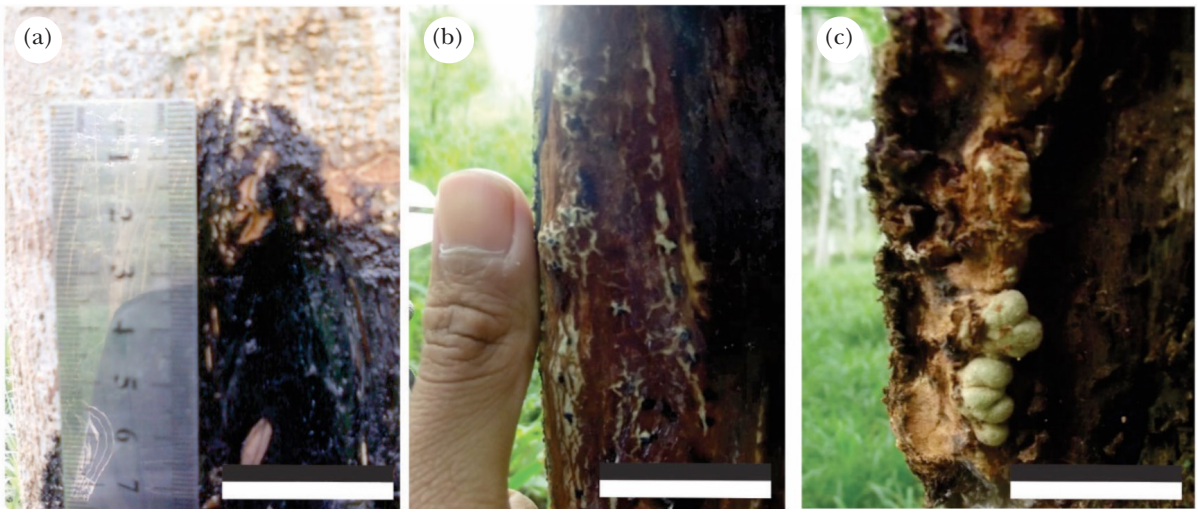

Figure 2 Gall reformation on *Falcataria moluccana* trunk, (a) gall upon TA1 tree (day 27), (b) gall upon TC2 tree (day 21), (c) gall upon TC3 tree (day 15); scale bar = 3 cm; TA = one time tar smearing, TC = without tar smearing

Removing gall and tar smearing treatment was not able to completely eliminate the effect of *U. tepperianum* infection, shown by the presence of fungus mycelia in wood tissue (Figure 3a). Tar smearing, conducted regularly, kept the existence of tar layer. Previous study showed that dominant environmental factors that could influence gall rust disease were fog intensity, relative humidity and wind speed (Rahayu 2014). Loss of tar layer caused by these environmental factors affected the development of *U. tepperianum* in wood tissue that enabled the occurrence of gall reformation.

Modification on proportion of wood cell type

Table 3 shows proportion of wood cell types with TA, TB and TC treated trees, where no significant change was observed. However, on several samples, high percentage of ray parenchyma and axial parenchyma was found. According to Ishiguri et al. (2009), percentage of ray parenchyma and axial parenchyma of

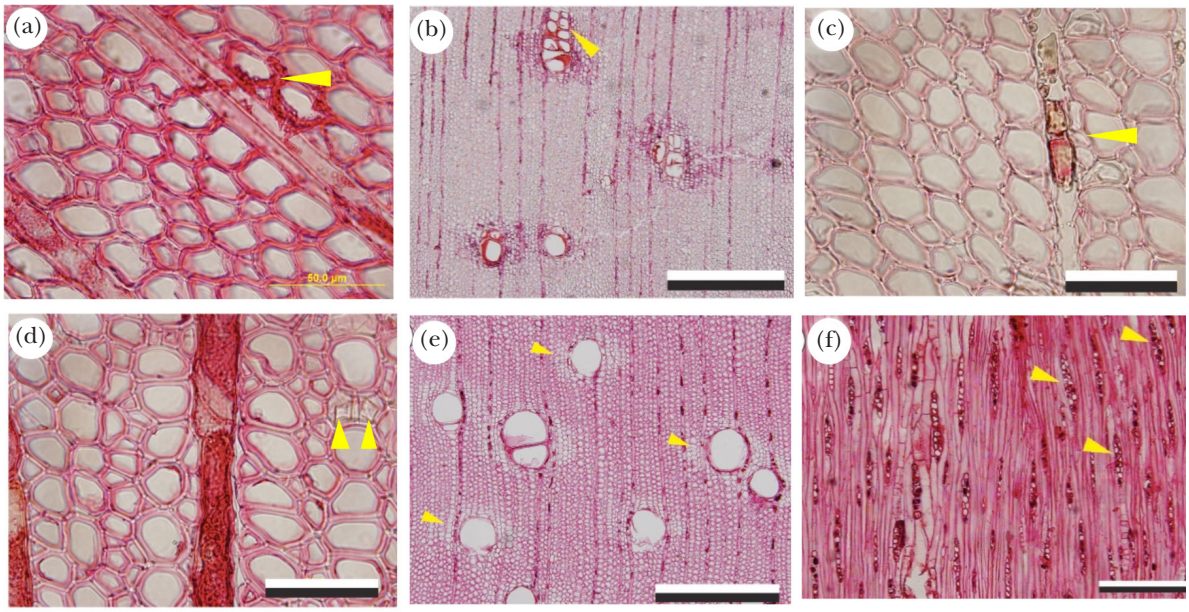

Figure 3 Sections of *Falcataria moluccana* wood attacked by gall rust disease, (a) = presence of mycelia (scale bar: 50 µm), (b) = vessel with cluster arrangement (scale bar: 500 µm), (c) = presence of phenolic-like compound (scale bar: 50 µm), (d) = presence of crystal (scale bar: 50 µm), (e) = lozenge-aliform axial parenchyma (scale bar: 500 µm), (f) = modification of ray (scale bar: 500 µm)

F. moluccana is 1–7% and 1–6% respectively. However, the highest percentage of ray parenchyma on TA, TB and TC treated trees were 10.5 ± 3.7%, 9.3 ± 1.7% and 9.1 ± 3.6% respectively, while the highest percentage of axial parenchyma were 8.6 ± 1.1%, 8.2 ± 6.5%, 8.0 ± 4.0% respectively. In a previous study, there was an increase of ray and axial parenchyma on wood tissue located beneath the gall part compared to wood tissue on the opposite part (Rukhama & Nugroho 2014). The percentage increase of ray parenchyma cells seems to be associated with high radial growth, similar to tumor wood in *Pinus densiflora* (Eom & Chung 1994). Similar response also occurred on *R. communis,* infected by crown gall disease in which the ray parenchyma size increased in the area close to the center of the gall (Aloni et al. 1995). The percentage of axial parenchyma was also found to be higher than normal *F. moluccana* wood (Ishiguri et al. 2009). The higher percentage of axial parenchyma might be related to the modification of axial parenchyma configuration (Figure 3e).

Structure of wood cell type

Generally, the vessels of *F. moluccana* are distributed in radial multiples, consisting of 2–3 vessels (Mandang & Pandit 1997). However, as seen in Figure 3b, there was a modification of vessel distribution into clusters. The tangential diameter of the vessels also became narrower. Diameter of vessels in treatments TA, TB and TC were 90.4 ± 42.3 to 143.7 ± 39.2 µm, 112.9 ± 16.9 to 170.9 ± 20.1 µm and 106.1 ± 12.9 to 153.0 ± 22.5 µm respectively. The result of this observation was smaller than normal *F. moluccana* which ranges from 140–286 µm (Ishiguri et al. 2009). Similarly, *R. communis* infected by crown gall disease had narrower vessel tangential diameter at the affected part (Aloni et al. 1995). Narrower vessels were also found in abnormal xylem of *Fraxinus mandshurica* attacked by *Nectria galligena* (Sakamoto et al. 2004). In ray parenchyma cell, there was presence of phenolic-like compound (Figure 3c) which shows that gall rust seems to spark the synthesis of phenolic. Synthesis of phenolic in plant is the defense response of plant towards pathogen (Agrios 1998).

Commonly, *F. moluccana* has uniseriate rays, one cell wide at most (Insidewood 2004, Wheeler 2011). However, Figure 3f shows the differed ray type on *F. moluccana* wood that is multiseriate. In crown gall disease that attacks *R. communis*, uniseriate ray type is modified to multiseriate (Aloni et al 1995). In addition, on *Pinus takahasii*

Table 3 Proportion of cell type

Cell type (%)		p	Day									
			Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 21	Day 27	Day 34	Day 41
Wood fibre												
	TA	0.796 ns	80.5 ± 2.6	83.3 ± 4.6	79.3 ± 4.5	83.4 ± 9.1	77.4 ± 4.0	80.3 ± 10.9	86.1 ± 6.9	79.1 ± 5.6	84.3 ± 0.9	81.9 ± 4.3
	TB	0.978 ns	82.1 ± 5.3	79.0 ± 5.1	83.9 ± 5.2	81.8 ± 7.4	83.5 ± 4.9	79.8 ± 9.0	81.0 ± 5.8	82.7 ± 5.5	83.8 ± 1.6	81.2 ± 4.1
	TC	0.402 ns	81.0 ± 6.5	84.0 ± 4.5	79.3 ± 3.6	82.1 ± 1.1	80.1 ± 6.3	84.2 ± 2.0	82.1 ± 2.2	77.4 ± 5.8	85.0 ± 0.1	83.2 ± 1.9
Ray												
	TA	0.401 ns	6.7 ± 2.3	7.52 ± 0.3	9.2 ± 2.0	9.5 ± 3.6	7.8 ± 1.1	8.1 ± 2.6	7.0 ± 1.6	10.5 ± 3.7	10.1 ± 2.3	6.3 ± 2.0
	TB	0.759 ns	7.7 ± 1.1	6.4 ± 1.3	6.4 ± 1.3	7.4 ± 2.2	7.1 ± 3.1	7.2 ± 2.0	9.3 ± 1.7	8.4 ± 2.1	7.0 ± 1.6	6.4 ± 1.5
	TC	0.562 ns	9.1 ± 3.6	7.2 ± 1.5	7.4 ± 0.8	8.6 ± 2.1	7.2 ± 1.0	8.2 ± 1.4	6.7 ± 1.3	8.6 ± 0.4	6.2 ± 1.4	8.7 ± 2.7
Axial parenchyma												
	TA	0.728 ns	7.1 ± 0.9	5.1 ± 1.6	6.2 ± 3.6	4.9 ± 4.6	8.6 ± 1.1	7.1 ± 7.1	4.6 ± 4.1	5.4 ± 3.6	2.4 ± 1.1	7.0 ± 3.2
	TB	0.732 ns	4.9 ± 3.5	7.3 ± 2.3	3.7 ± 1.4	57 ± 3.5	5.0 ± 2.7	8.2 ± 6.5	4.9 ± 2.0	3.8 ± 2.3	3.9 ± 0.8	6.4 ± 3.0
	TC	0.055 ns	4.5 ± 1.9	3.7 ± 1.3	7.6 ± 0.8	5.0 ± 0.9	5.4 ± 2.6	3.7 ± 0.7	5.9 ± 2.1	8.0 ± 4.0	3.5 ± 0.4	3.8 ± 0.2
Vessel												
	TA	0.504 ns	5.8 ± 1.2	4.1 ± 3.9	5.3 ± 2.2	2.3 ± 1.6	6.2 ± 3.9	4.6 ± 1.6	2.3 ± 1.3	5.0 ± 2.4	3.3 ± 1.1	4.8 ± 1.0
	TB	0.861 ns	5.4 ± 1.1	7.3 ± 1.7	5.9 ± 2.5	5.1 ± 1.7	4.3 ± 0.7	4.9 ± 1.0	4.8 ± 2.8	5.1 ± 2.8	5.4 ± 1.1	6.0 ± 2.5
	TC	0.816 ns	5.5 ± 1.5	5.2 ± 2.3	5.8 ± 4.2	4.3 ± 2.5	7.3 ± 4.2	3.9 ± 0.9	5.3 ± 0.1	6.1 ± 2.2	5.3 ± 1.4	4.4 ± 0.8

ns = non significantly different at $p < 0.05$; TA = one time tar smearing, TB = per 15 days tar smearing, TC = without tar smearing

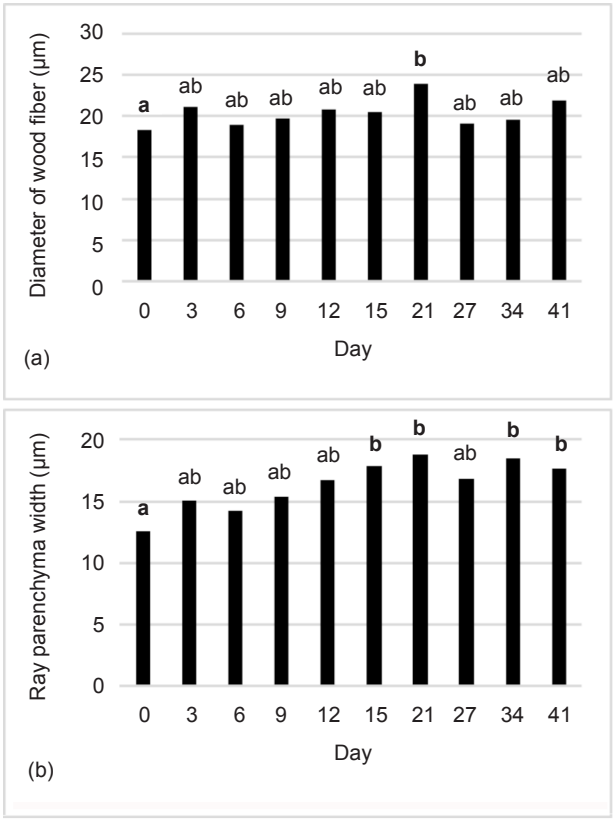

Figure 4 Measurement of (a) diameter of wood fiber and (b) ray parenchyma width on *Falcataria moluccana* treated with one time smearing; different letters above the bars indicate significant differences, as determined by Tukey's post hoc test, at $p < 0.05$

attacked by *C. quercuum*, the ray width of infected tissue was wider than healthy tissue (Liping et al. 1990).

Falcataria moluccana has axial parenchyma configuration that consists of diffuse, vasisentric and scanty (Chauhan & Dayal 1985, Insidewood 2004, Wheeler 2011, Martawijaya et al. 1989). However, Figure 3f shows another configuration of axial parenchyma that is lozenge-aliform on several *F. moluccana* wood samples.

The presence of crystal substance in wood tissue can be seen in Figure 3d but this substance was not found in gall tissue (Rukhama & Nugroho 2014). This indicated that the distribution of water and nutrition substance was not disturbed by gall rust disease. In contrast, on crown gall that developed on *R. communis*, abundant crystal substance was found in gall tissue, showing the higher ability of water and nutrient absorption than healthy host tissue (Aloni et al 1995).

Morphology of wood cell type

Several morphological wood cell types are shown in Table 4 where significant increase occurred in fibre diameter of TA treatment. On day 0, fibre diameter was 18.4 ± 1.6 µm, which increased to 24.0 ± 1.3 µm on day 21 (Figure 4). Significant increase was also found in the width of ray parenchyma for the same treatment. The width of ray parenchyma, day 0 to 15, increased from 12.6 ± 1.03 µm to 17.9 ± 2.7 µm respectively (Figure 4). The result seems to be associated with the gall reformation upon TA tree observed on day 21. Loss of tar layer could not protect the inside of *F. moluccana* trunk from environmental factors, thus the *U. tepperianum* developed inside the wood. This caused modification on wood anatomy followed by tumor reformation upon the trunk. However the TC treated samples which were not smeared with tar did not show significant modification on morphology of xylem cells. Individual host did not support the rapid development of fungus on modifying xylem cells.

CONCLUSIONS

Tar treatment significantly reduced gall reformation which was shown by the absence of gall reformation on *F. moluccana* trees that were smeared by tar regularly. Tar smearing had to be conducted every 15 days to get satisfactory results. Significant modifications on wood anatomical properties occurred on TA i.e. wood fibre diameter and ray width. Modification of xylem cells anatomy took place when tar smearing treatment was not frequently conducted. The effects of gall rust on *F. moluccana* xylem cells could be observed through the appearance of fungi mycelia in fibre cell, formation of multiseriate ray cells, the presence of phenolic-like compound and modification on axial parenchyma cell arrangement, as well as narrower vessel element.

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Table 4 Morphology of wood cell type

Anatomical property (µm)		p	Day									
			Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 21	Day 27	Day 34	Day 41
Wood fiber diameter												
	TA	0.044 *	18.4 ± 1.6 a	21.1 ± 1.5 ab	19.0 ± 0.8 ab	19.8 ± 1.9 ab	20.7 ± 1.5 ab	20.5 ± 3.8 ab	24.0 ± 1.3 b	19.1 ± 1.2 ab	19.7 ± 1.7 ab	22.0 ± 1.3 ab
	TB	0.369 ns	18.2 ± 1.7	25.5 ± 4.1	19.4 ± 4.2	19.8 ± 3.6	19.7 ± 4.3	22.5 ± 4.8	17.8 ± 2.7	22.2 ± 4.8	20.3 ± 3.3	22.3 ± 2.8
	TC	0.233 ns	21.6 ± 0.9	22.0 ± 3.3	18.0 ± 2.1	22.6 ± 0.6	22.3 ± 1.3	22.0 ± 1.9	21.5 ± 4.2	23.2 ± 1.9	24,4 ± 1.9	22.35 ± 2.9
Vessel diameter												
	TA	0.871 ns	141.8 ± 30.7	135.4 ± 23.8	129.9 ± 45.2	90.4 ± 42.3	130.0 ± 46.0	125.6 ± 55.1	107.3 ± 47.8	111.8 ± 41.9	132,0 ± 37.4	143.7 ± 39.2
	TB	0.780 ns	152.3 ± 16.6	170.9 ± 20.1	167.4 ± 17.6	134.2 ± 26.8	112.9 ± 16.9	151.2 ± 43.6	135.6 ± 45.6	141.9 ± 76.5	144.2 ± 29.6	139.2 ± 37.4
	TC	0.794 ns	106,1 ± 12.9	153.0 ± 22.5	124.7 ± 68.4	149.2 ± 36.6	139.9 ± 18.5	123.4 ± 40.1	148.0 ± 18.0	112.6 ± 22.3	121.5 ± 56.0	135.0 ± 27.7
Ray width												
	TA	0.004 **	12.6 ± 1.03 a	15.1 ± 1.5 ab	14.3 ± 2.0 ab	15.3 ± 0.8 ab	16.7 ± 1.4 ab	17.9 ± 2.7 b	18.8 ± 1.4 b	16.8 ± 0.7 ab	18.5 ± 3.1 b	17.7 ± 0.9 b
	TB	0.105 ns	14.4 ± 2.3	13.4 ± 1.4	15.6 ± 2.5	16.0 ± 1.5	16.4 ± 0.9	14.2 ± 0.9	16.7 ± 0.4	15.1 ± 2.0	17.5 ± 1.6	16.1 ± 1.3
	TC	0.113 ns	13.8 ± 2.1	13.5 ± 2.1	17.5 ± 1.7	15.3 ± 1.2	15.5 ± 1.5	17.4 ± 0.9	15.7 ± 1.8	15.4 ± 2.3	15.9 ± 0.3	14.9 ± 1.3
Ray height												
	TA	0.875 ns	217.8 ± 33.0	170.9 ± 48.7	217.0 ± 64.8	190.5 ± 30.3	210.2 ± 16.8	192.1 ± 17.3	192.5 ± 45.6	186.0 ± 20.6	195.6 ± 22.6	199.4 ± 34.5
	TB	0.191 ns	220.8 ± 16.4	190.9 ± 22.5	208.3 ± 18.0	202.7 ± 7.3	196.0 ± 17.6	164.6 ± 34.2	203.7 ± 24.7	171.4 ± 14.2	201.8 ± 41.1	199.9 ± 15.7
	TC	0.528 ns	160.9 ± 39.2	193.8 ± 52.5	228.5 ± 68.2	175.0 ± 33.2	154.0 ± 22.0	200.7 ± 55.6	185.2 ± 18.4	197.2 ± 30.1	170.9 ± 27.1	173.4 ± 8.3

Different letters (a, ab, b) = significantly different at $p < 0.05$, Tukey *post hoc test,* ns = non significantly different at $p < 0.05$, * = significantly different at $p < 0.05$, ** = significantly different at $p< 0.01$; TA = one time tar smearing, TB = per 15 days tar smearing, TC = without tar smearing

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