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Original Article

Evaluation of incense-resinous wood formation in agarwood (*Aquilaria malaccensis Lam.*) using sonic tomography

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ABSTRACT

Incense-resinous wood of agarwood is a high-value non-timber forest product found in the trunk or branches of *Aquliaria* and *Gyrinops* species. Incense-resinous wood of agarwood is formed as a response to tree damage caused by wounding or fungal attack. Detection of such wood in trees has generally been carried out based on natural signs such as dark spots or black marks when peeling back tree bark, but these often yield uncertain results. Sonic tomography can be applied to predict the presence of incense-resinous wood in standing trees. The objective of this study was to evaluate sonic tomography at various trunk heights based on variations in the sound velocity associated with the presence of incense-resinous wood. Ten agarwood trees (*Aquilaria malaccensis*) were selected for this study; five trees were artificially inoculated with *Fusarium solani* fungus and the other five were untreated. The results showed that the height of the measurement did not significantly affect the propagation velocity of sound waves or the tomographic results. Sonic tomography revealed that prediction of the deteriorated zone which is indicative of incense-resinous wood formation was 1.1% greater in inoculated trees than in uninoculated trees.

Introduction

Incense-resinous wood of agarwood is a high-value, non-timber forest product found in the trunk or branches of some tree species belonging to the genera *Aquilaria* and *Gyrinops* and it appears within the woody tissues of the trees as chunks or dark clumps that contain an aromatic resin, and when it is burned, it produces a fragrant odor (Sitepu et al., 2010). Incense-resinous wood forms in the living woody tissue of trees in response to natural or artificial fungal attack, injury or other non-pathological processes that induce changes in the physiological and chemical compounds of the wood (Groves and Rutherford, 2015). Because of its pleasant, unique aroma, incense-resinous wood of agarwood is used as a raw material in the manufacture of perfumes, cosmetics and traditional medicines (Kim et al., 1997; Bhuiyan et al., 2009). Compared with other forest products, incense-resinous wood of agarwood has a high economic value (Siran and Turjaman, 2010); thus it is intensively sought. As a result, the survival of agarwood-producing trees species, including *Aquilaria malaccensis* Lam., is seriously threatened. Since 1994, *Aquilaria* spp. have been listed as endangered tree species in Appendix II of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (2004). Consequently, harvesting and export of incense-resinous wood of agarwood are controlled and restricted to certain quotas (Convention on International Trade in Endangered Species of Wild Flora and Fauna, 2004).

Collection of incense wood from agarwood has traditionally relied on detecting natural signs visible on tree trunks, mainly spots or black marks on the wood when the bark is removed. Trees with these signs are thought to contain incense wood and are then harvested. The process can be characterized as trial and error, however, because the agarwood trees are often felled without the certainty that they contain incense wood. Chua (2008) reported two harvesting methods for incense wood in agarwood trees: fatal harvest and sub-lethal harvest. Fatal harvest is the most common, and it involves the whole tree being chopped down to harvest the incense wood, while sub-lethal harvest involves harvesting the incense wood of agarwood after several years of coppicing.

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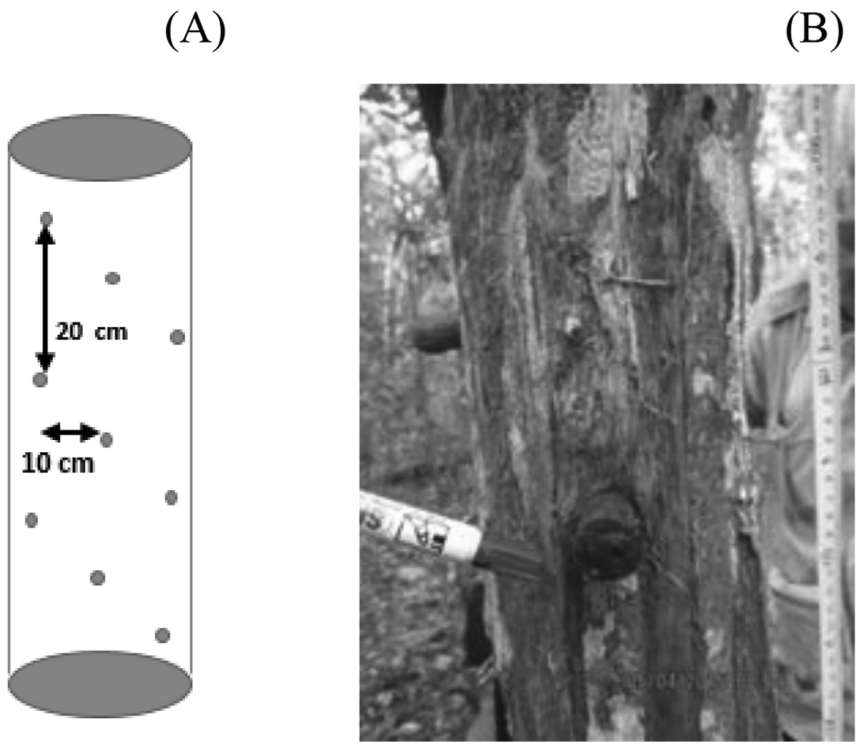

Fig. 1. Inoculation process on agarwood trunk: (A) Pattern of fungal inoculation holes in the tree trunk; (B) Wooden peg used to plug the inoculation hole.

Current non-destructive testing techniques based on sound waves paired with imaging technology (tomography) use the sound wave propagation velocity to create a color image of the inside of the material, such as a tree trunk (Gilbert and Smiley, 2004; Wang et al., 2008; Lin et al., 2011; Li et al., 2014). Several studies have used sonic tomography to detect the presence of defects or to assess the condition of the inside of tree trunks (Liang et al., 2007; Wang et al., 2008; Li et al., 2014). A study of incense wood in the agarwood-producing tree species *A. microcarpa* (Indahsuary et al., 2014) demonstrated the reliability and potential of non-destructive testing techniques by using a PiCUS sonic tomography instrument to identify incensed wood in a tree stand. Ahmad et al. (2012) reported that the sound wave velocity is lower in deteriorated areas containing incense agarwood than in areas that do not contain

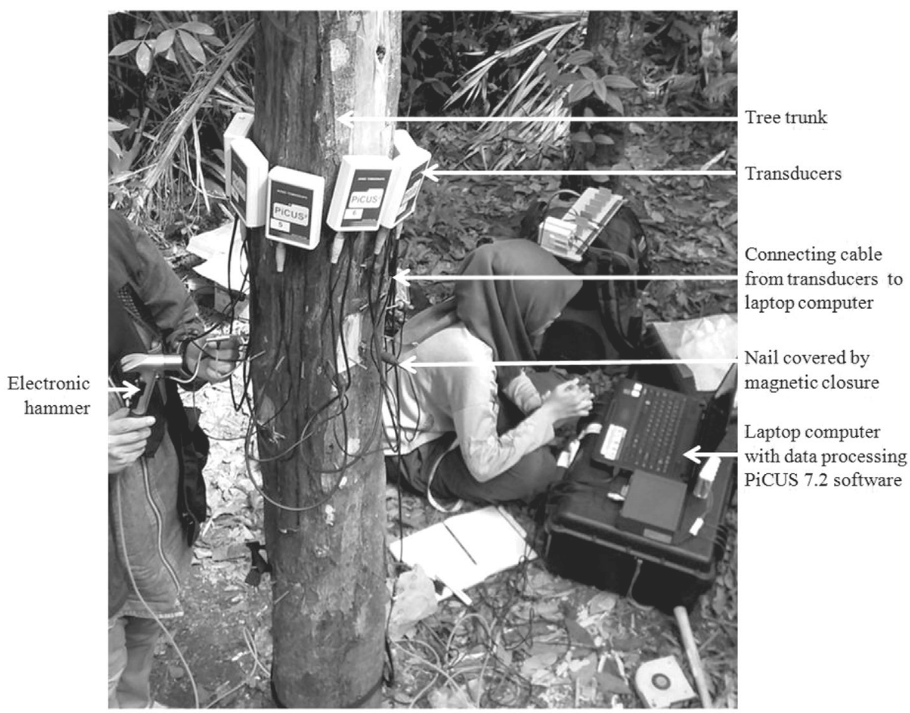

Fig. 2. Application of sonic tomography on agarwood tree trunk.

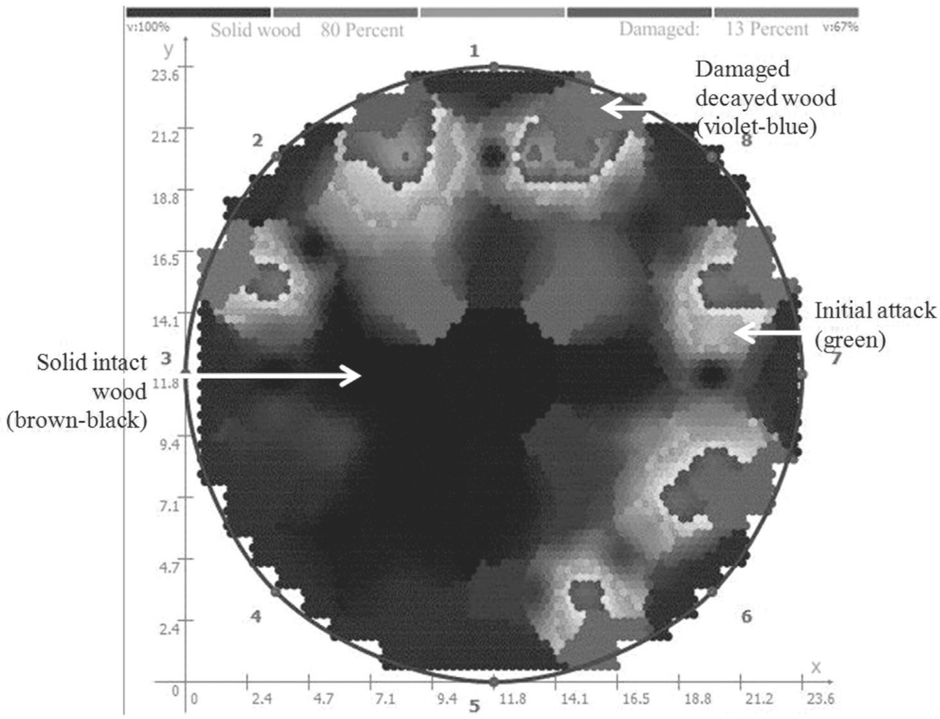

Fig. 3. Internal condition of the wood as shown by a tomogram. The full spectral color range is shown in grayscale here with solid wood and damaged wood as well as initial attack indicated by the arrows.

incense wood. Studies using acoustic tomography have shown that tomograms obtained at various heights along a tree trunk depend on the continuity of irregularity caused by defects in the wood (Wang et al., 2008; Wang and Allison, 2008; Kazemi et al., 2009; Li et al., 2014). Tomograms at several heights typically generate interconnected patterns. Given that studies on the axial distribution of agarwood in tree trunks are limited, the aim of the present study was to evaluate sonic tomography with respect to the distribution of incense wood formation in the axial direction based on the sound wave velocity.

Materials and methods

Ten 11-year-old agarwood (*A. malaccensis Lam.*) trees with a diameter of at least 20 cm, located in community forests of Prabumulih, South Sumatra province, Indonesia, were used in this study. Five agarwood trees had been induced to produce incense-resinous wood via inoculation with Jambi *Fusarium solani* strains (FORDA CC00500) 30 mth before the testing began. The remaining five agarwood trees were untreated or uninoculated. Selection of induced trees was based on the visual inspection of their trunks and information from the landowners. The induction process was done by drilling holes at set intervals in an ascending spiral pattern around a tree trunk, from the bottom to the top of the tree (Fig. 1A). Drill holes were approximately 0.8 mm in diameter, and their depth was 1/3 of the tree diameter. Fungal isolates were injected into the holes, which were then plugged with wooden pegs (Fig. 1B).

Non-destructive testing was conducted by using a PiCUS sonic tomograph (Argus-Electronic Gmbh; Rostock, Germany). Sensors or transducers were mounted around the trunk at six distances above ground level at 20 cm, 70 cm, 120 cm, 170 cm, 220 cm and 270 cm. Generally, 7–12 transducers per tree were used, depending on the tree diameter. The first transducer was placed at the north position in order to facilitate verification of the testing location. One of the transducers sent a sound wave signal, while another sensor served as a receiver. Sound waves were generated by using an electronic hammer to tap on a nail that was attached above or below each transducer. These nails were connected to the transducer and the computer device. When one of the nails was being tapped, the others were covered using a magnetic closure to avoid wave propagation that would bias the results (Fig. 2). The resulting sound wave velocities were then further processed using the PiCUS Q7.2 software (Argus-Electronic Gmbh; Rostock, Germany), which converted the data into colored tomograms, which could be viewed on the laptop computer.

The tomogram reflected sound wave propagation velocity data and provided information about wood deterioration, including the percentage of the wood that was either in a state of advanced decay or intact (Fig. 3). The area between the deteriorated and intact zones was defined as an intermediate zone. The intact zones

Table 1
Tree dimensions in inoculated and uninoculated agarwood (*A. malaccensis*) trees.

	Diameter (cm)		Height (m)		Volume (m^3)	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
Average	18.66	21.34	12.6	11.9	0.25	0.31
Maximum	20.06	26.11	14.0	14.0	0.31	0.53
Minimum	17.20	17.83	7.0	11.0	0.11	0.19
SD	1.23	3.13	3.03	1.24	0.08	0.31

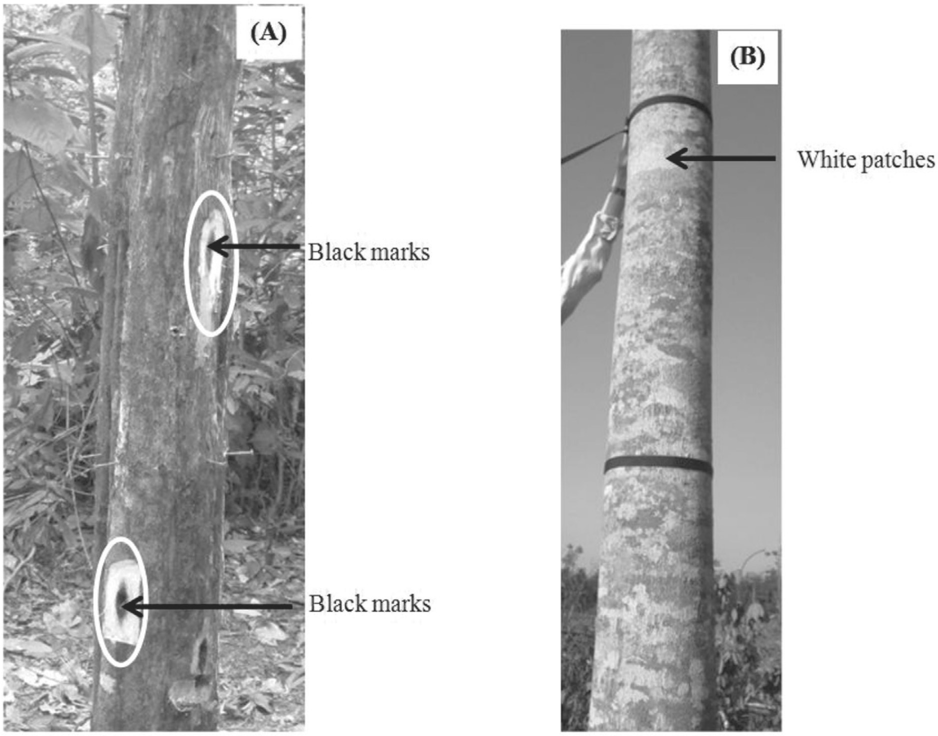

Fig. 4. Black marks on an inoculated tree at several heights along the trunk (A); Uninoculated tree with white patches of fungus (B).

were characterized by a dark color (brown-black) on the tomogram, which represented sound waves with a high velocity. The intermediate zones were characterized by a green-to-violet color, which indicated early-to-moderate attack from the organisms causing the wood weathering. Finally, a bluish color indicated advanced decay or holes, which slowed the propagation of sound waves (Göcke et al., 2010).

Results and discussion

Tree condition

Data on the dimensions of inoculated and uninoculated trees are presented in Table 1. *Aquilaria* spp. are fast growing and grow well at an altitude up to about 500 m above sea level in areas with an average daily temperature of 20–22 °C (Akter et al., 2013). Suhartati and Wahyudi (2011) reported that most *A. malaccensis* trees cultivated in community forests grow well in rocky, podzolic soil with a sandy-clay-loam texture at elevations ranging from 10 m to 400 m above sea level, with an air temperature of 24–32 °C. Under these conditions, the trees can reach a height of 4.8 m and a trunk diameter of 7.1 cm at age 4 yr. Mohamed et al. (2013) categorized 3-year-old planted *A. malaccensis* as old saplings, being 2 m tall and 4 cm in diameter, which would yield juvenile wood. Most agarwood-producing tree species require shade during the early sapling phase (Sumarna, 2002).

Visual observations of inoculated tree trunks involved peeling back tree bark to find black marks or spots present at several heights along the trunk, which indicated the formation of agarwood (Fig. 4A). These findings were consistent with the results of previous studies. For example, a study on cultivated *A. malaccensis* tree by Chong et al. (2015) showed that incense-resinous wood forms in tree trunks 18 mth after fungal inoculation. Pojanagaroon and Kaewrak (2005) reported that mechanical wounding caused incense-resinous wood to start forming in 4-year-old *A. crassna* tree trunks after 3 mth, which was marked by a color change from pale-brown to yellow-brown. The color became light brown at 8–10 mth and turned black after 20 mth. Uninoculated trees were found to have white patches of fungus along the tree (Fig. 4B). Persoon (2007) reported that incense-resinous wood could form in uninoculated trees in approximately 15 yr.

Sound wave propagation velocity

The average value of the sound velocity was in the range 809–934 m/s for inoculated agarwood trees, while for uninoculated trees the value was in the range 799–875 m/s (Table 2). The highest velocity for sound wave propagation was obtained at a height of 20 cm in both inoculated and uninoculated agarwood trees. However, single-factor statistical analysis showed that height did not significantly affect the sound wave velocity ($\alpha = 5\%$), and a comparison test (Student's *t*-test) revealed no significant difference with respect to inoculated and uninoculated agarwood trees ($p > 0.05$). The uniform condition of the tree trunks associated with the spiral pattern of the inoculation technique allowed the spread of fungal infection to be the same at every height. The sound wave velocity in uninoculated agarwood trees compared to inoculated

Table 2
Tree sound wave velocity values based on distance from the ground (height).

Height from above the ground (cm)	Average sound wave propagation velocity (m/s)	
	Inoculated tree	Uninoculated tree
20	934	875
70	877	864
120	915	862
170	877	799
220	809	786
270	881	781
Average	882	828
SD	42.83	43.53

Table 3
Mean values of tomogram image zone in sonic tomographs.

Trunk height (cm) ($n = 5$)	Inoculated*				Uninoculated			
	% So	*% Dm*	*% Im*	Deteriorated zone (%) ($Dm + Im$)	*% So*	*% Dm*	*% Im*	Deteriorated zone (%) ($Dm + Im$)
20	85.8	5.6	8.6	14.2	92.6	0.8	6.6	7.4
70	89.0	2.0	9.0	11.0	88.0	2.4	9.6	12.0
120	87.0	4.6	8.4	13.0	88.4	3.6	8.0	11.6
170	89.2	2.6	8.2	10.8	90.8	3.6	5.6	9.2
220	91.0	2.8	6.2	9.0	89.4	3.8	6.8	10.6
270	90.2	3.8	6.0	9.8	89.4	0.8	9.8	10.6
Average	88.7	3.6	7.7	11.3	89.8	2.5	7.7	10.2

*So = solid zone, Dm = damage zone, Im = intermediate zone.

agarwood trees was only slightly higher, suggesting that deteriorated wood was also present in uninoculated agarwood trees. The white fungal patches in these trees (Fig. 4B) were presumably evidence that they had begun to deteriorate. Furthermore, uninoculated tree tomograms depicted the green-to-violet color in the timber cross section that denoted deteriorated wood.

The sound wave velocity in tree trunks attacked by brown-rot fungus ranged between 550 m/s and 1250 m/s, as reported by Li et al. (2014). Deflorio et al. (2008) reported that the sound wave velocity in some temperate trees, such as oak (*Quercus robur* L.), sycamore (*Acer pseudoplatanus* L.), beech (*Fagus sylvatica* L) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) that were inoculated using several fungi 27 mth before testing, decreased about 10%, based on tomographic images; the type of fungus determined the wood deterioration rate. Karlinasari et al. (2015) reported that the sound wave velocity in *Aquilaria microcarpa* trees containing incense-resinous wood was in the range 529–807 m/s for sonic waves and in the range 807–1203 m/s for ultrasonic waves.

A tomogram study conducted by Kazemi et al. (2009) at various heights (100 cm, 430 cm and 800 cm) above ground level showed that higher measurement points tended to have lower sound wave velocities. Wang et al. (2008) and Li et al. (2014) reported that the tomographic images had more colors at a height of 50 cm above the ground, indicating widespread decay; at greater heights, the extent of decay became progressively less. Wang and Allison (2008) tested

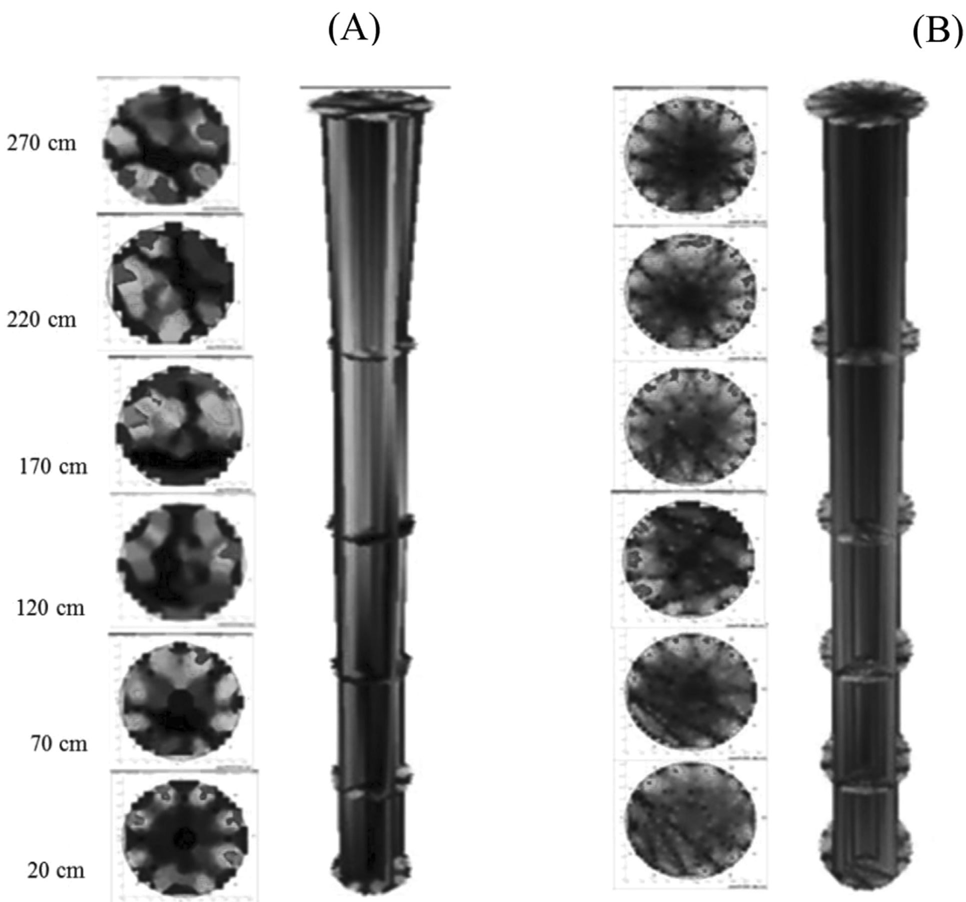

Fig. 5. Example of two-dimensional and three-dimensional tomograms at several heights along the agarwood trunk: (A) inoculated tree P1; (B) uninoculated tree P6. (darker areas indicate solid or intact wood, while lighter areas indicate initial attack).

the reliability of acoustic technology in tree trunks at 10 cm, 100 cm and 200 cm above the ground. They reported some cracks on the side of trunks at 10 cm and 100 cm, while visual observation showed fewer defects at 200 cm than at the other heights. Indahsuary et al. (2014) and Karlinasari et al. (2015) reported that the sound wave velocities at 20 cm, 130 cm and 200 cm above ground were not significantly different in inoculated *A. microcarpa* trees.

Tomographic image results

The tomographic images in this study showed that the average percentage of solid zone was 88.7% in inoculated agarwood trees and 89.8% in uninoculated trees. The average percentages of damaged zones in inoculated and uninoculated agarwood trees were 3.6% and 2.5%, respectively (Table 3). The deteriorated zone (damaged and intermediate) in inoculated agarwood trees was 1.1% higher compared to uninoculated trees.

Light-colored areas in Fig. 5A indicate deteriorated and intermediate zones and were larger in inoculated trees than in uninoculated trees (Fig. 5B). This suggests that inoculated trees contained more deteriorated wood than uninoculated trees. Green-to-violet color in the tomograms of inoculated trees revealed earlier fungal attack that was more severe than in uninoculated trees. It occurred along the trunk height and seemed to extend inward into the tree. In uninoculated trees, the deteriorated zones as shown by the tomograms were only found in the outer part of the tree trunk.

Several factors affect the formation, quantity and quality of incense-resinous wood production, including the species of tree, the species and purity of the microorganism, the suitability of inoculants under existing conditions, the use of superior inoculants, the inoculation technique and the time between inoculation and incense-resinous wood collection (Turjaman et al., 2016). In general, the longer the time between inoculation and harvest, the higher the quality of agarwood produced (Mucharromah and Santoso, 2008; Chen et al., 2011). Other factors such as the age of the tree and differences caused by environmental variations and genetic variations in *Aquilaria* spp. also play important roles in incense-resinous wood formation (Ng et al., 1997).

Incense-resinous wood that forms naturally begins with wounding, such as from a broken branch or a breach in the bark. This wounding allows the entry of microorganisms, including *Fusarium* spp., which spread to the inner trunk. The fungal infection is characterized by patches of wood tissue that is dark brown in color. The yield of incense-resinous wood that occurs naturally is very low, around 30%, and its formation may require approximately 15 yrs; therefore, artificial inoculation techniques are needed to meet the demand for agarwood (Persoon, 2007).

Conclusion and recommendation

These findings indicate that tomography could be useful for assessing incense-resinous wood formation in inoculated trees. The height of the measurement point on the *Aquilaria malaccensis* trunk did not significantly affect the velocity of sound waves generated by a sonic tomography instrument. Moreover, tomographic images showed an identical color gradation pattern at all heights. Use of this tool could reduce premature or wasteful harvest of *A. malaccensis* trees that do not contain agarwood.

Conflict of interest

There is no conflict of interest.

Acknowledgments

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