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SHORT COMMUNICATION

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Distribution of *Rigidoporus lignosus* genotypes in a rubber plantation, as revealed by somatic compatibility

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Abstract A study was conducted to determine the spatial distribution of somatic incompatibility groups (SIGs) in *Rigidoporus lignosus* populations responsible for white root disease in a south Sumatera rubber plantation. Most isolates colonizing the stumps were somatically compatible with isolates from either a nearby stump or a living rubber tree, which suggested evidence of stump-to-tree or stump-to-stump clonal growth of the fungus. SIGs of *R. lignosus* occupied an area territorially in which the minimum size estimate for the largest genotype was 25 m in length.

Key words Clonality · *Rigidoporus lignosus* · Somatic incompatibility · Rubber tree

Rigidoporus lignosus (Klotzsch) Imazeki is the most economically important fungal pathogen in rubber and timber production in the tropics, especially in Indonesia, Malaysia, Sri Lanka, and the Ivory Coast (Liyanage 1997; Semangun 2000). The fungus attacks the roots and collar region of the taproot, causing white root disease, and eventually kills trees at any growth stage. Management of the disease in Indonesia and other tropical regions has been developed without any knowledge of the population biology of the causal fungus. Studying population biology is the most important prerequisite to develop a sustainable strategy and tactics in management of plant disease (McDonald 1997). However, no studies have been reported concerning the population structure of *R. lignosus* in any countries, including Indonesia.

Studying fungal population biology requires appropriate markers for identifying individual genotypes (clones or genets) in a population of the fungus (Anderson and Kohn 1995). Somatic incompatibility has been reported to be used successfully as a marker for identification of individual genotype in polypore fungi, i.e., *Phellinus* (Hansen and Gohen 2000) and *Ganoderma* (Adaskaveg and Gilbertson 1987; Rolph et al. 2000). The objective of this study was to determine somatic incompatibility groups (SIGs) in field isolates of *R. lignosus* and to describe their spatial distribution in a rubber plantation.

Study sites consisted of two sites of the white root disease foci within a 15-ha plantation replanted with rubber (*Hevea brasiliensis*) clone PR300, in 1995, in Sembawa, South Sumatera, Indonesia. In this study, a total of 17 basidiomes of *R. lignosus*, of which 9 were collected from site A in November 1999 and the rest from site B in March 2002, were used. The disease foci of site B are located 1200 m south of site A. Isolates were obtained from basidiomal context plated on glucose yeast extract agar (GYEA; 1% glucose, 0.2% yeast extract, and 2% agar) supplemented with 150 μl/ml H_2O_2 after autoclaving and maintained on the same medium without H_2O_2 at room temperature.

To determine SIG, we paired isolates collected from the same sites in all possible combinations. Intersite pairings could not be performed because contamination destroyed all isolates from site A. Fungal isolates were paired 1 cm apart on a thin layer of GYEA in a Petri dish. Self-crosses were performed as controls. Each pairing was repeated four times. After 4- to 8-week incubation at 28°C, macroscopic mycelial interactions were examined. The interaction was determined as incompatible when paired mycelia produced a lysis zone (reduced growth and/or dark pigment deposition along the zone of interaction). In contrast, the absence of a discernible lysis area was evident in compatible pairings that indicated similar or identical genetic constitution of the paired isolates. The isolates, which were somatically compatible to each other, were therefore grouped as the same SIG.

Of 36 pairings tested for site A, 4 pairings (Sbs1 × Sbs2, Sbs3 × Sbt4, Sbs5 × Sbt6, and Sbs7 × Sbs8) showed compatible interaction, where paired isolates grew together and

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Table 1. Somatic interactions among isolates of *Rigidoporus lignosus* paired on glucose yeast extract agar

SIG		Isolates collected from site A								
		Sbs1	Sbs2	Sbs3	Sbt4	Sbs5	Sbt6	Sbs7	Sbs8	Sbt9
A-1	Sbs1	/								
A-1	Sbs2	+	/							
A-2	Sbs3	=	=	/						
A-2	Sbt4	=	=	+	/					
A-3	Sbs5	=	=	=	=	/				
A-3	Sbt6	=	=	=	=	+	/			
A-4	Sbs7	=	=	=	=	=	=	/		
A-4	Sbs8	=	=	=	−	=	=	+	/	
A-5	Sbt9	=	=	=	=	=	=	−	=	/
		Isolates collected from site B								
		Sbs10	Sbs11	Sbs12	Sbs14	Sbs13	Sbs16	Sbs15	Sbs17	
B-1	Sbs10	/								
B-2	Sbs11	−	/							
B-2	Sbs12	−	+	/						
B-2	Sbs14	−	+	+	/					
B-3	Sbs13	−	−	−	−	/				
B-3	Sbs16	−	−	−	−	+	/			
B-4	Sbs15	−	−	−	−	−	−	/		
B-4	Sbs17	−	−	−	−	−	−	+	/	

SIG, somatic in compatability group; /, self-cross; =, incompatible interaction by formation of two zones of sparse aerial mycelia with brownish pigment production along colony junction; −, incompatible interaction with a zone of sparse aerial mycelia without pigmentation; +, compatible, where paired isolates grew together and merged to form a single colony

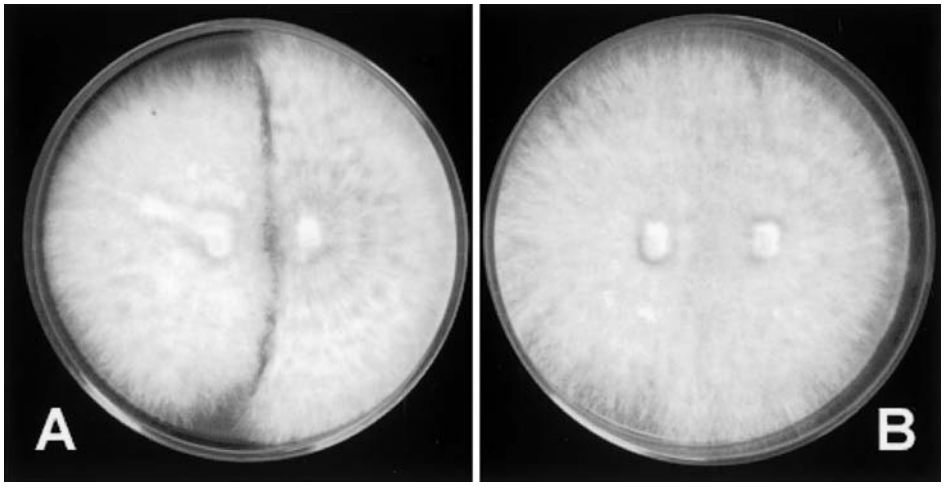

Fig. 1. Macroscopic somatic interactions between isolates of *Rigidoporus lignosus* paired on glucose yeast extract agar. **A** Somatically incompatible interaction with a zone of sparse aerial mycelia without pigmentation; **B** somatically compatible interaction where paired isolates grew together and merged to form a single colony

merged to form an apparently single colony. The similar result was observed in 5 compatible interactions (Sbs11 × Sbs12, Sbs11 × Sbs14, Sbs12 × Sbs14, Sbs13 × Sbs16, and Sbs15 × Sbs17) of 28 pairings between isolates from site B (Table 1). Pigmentation was not found in this somatically compatible interaction (Fig. 1B). These compatible interactions were macroscopically similar to those observed in self-pairings.

The rest of the paired isolates showed incompatible interactions by producing a clear zone with a thin line of submerged mycelium separating two mycelia. Most somatic interactions of isolates from site A were characterized by the formation of two zones of sparse aerial mycelia with brownish pigment production along the colony junction. Two pairings of isolates collected from site A (Sbt4 × Sbs8 and Sbs7 × Sbt9) and all isolates collected from site B showed weakly incompatible interactions with a zone of sparse aerial mycelia without pigmentation (Fig. 1A). Because we used the same cultural media in both pairing tests, it was likely that factors other than the medium could affect intensity of incompatible interaction. In *Phellinus gilvus*, relatedness between nuclei of paired mycelia affected in-

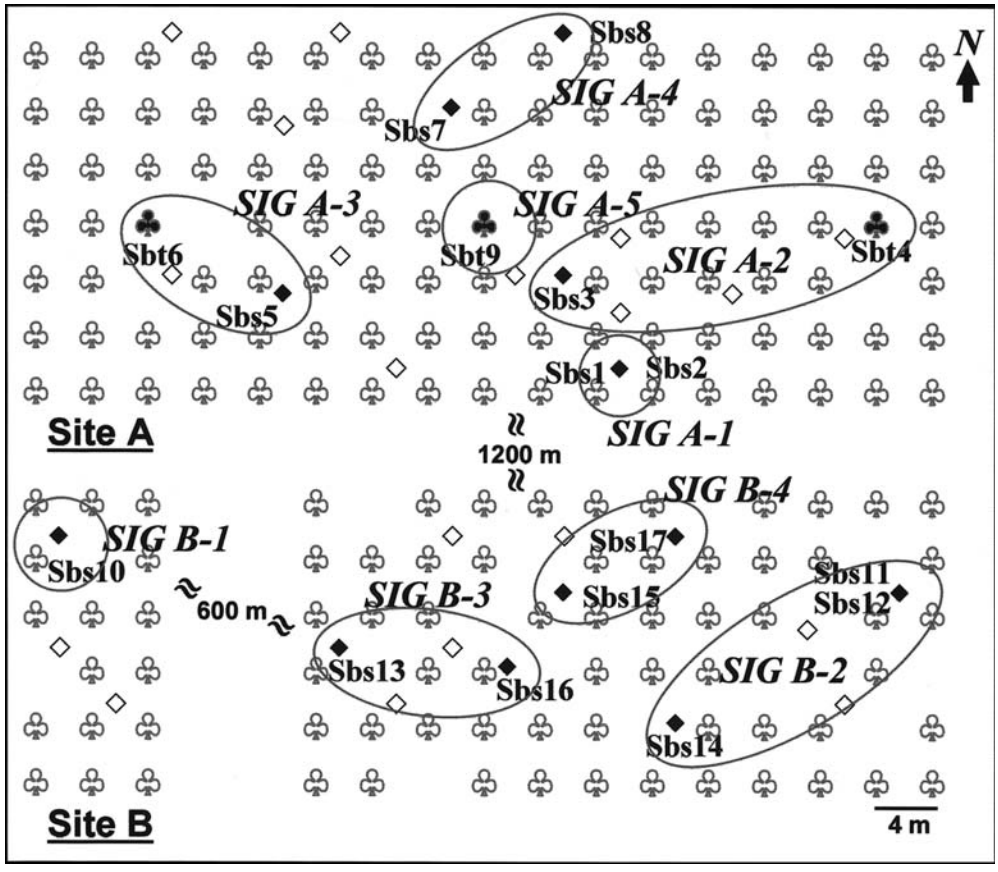

Fig. 2. Distribution map of somatic incompatibility groups (SIGs) of *Rigidoporus lignosus* existing in a rubber plantation. *Lines* enclose isolates of each SIG and are not intended to indicate the exact SIG boundaries; ♣, basidiomes on living rubber tree; ◆, basidiomes on stumps of rubber tree; ♧, living rubber tree without basidiome; ◇, stump without basidiome; *≈1200 m≈*, approximate distance between *site A* and *site B* is 1200 m; *≈600 m≈*, distance between isolate *Sbs10* and *Sbs 13* is about 600 m; *4 m*, row spacing is 4 m

compatible responses in which weak incompatibility was found only in pairing between sib-composed mycelia (Rizzo et al. 1995).

The isolates colonizing 4-year-old rubber trees were somatically compatible with isolates from their neighboring stumps (Sbs3 × Sbt4 and Sbs5 × Sbt6). This result suggested that isolates colonizing trees originated from the vegetative growth of isolates colonizing stumps. A similar growth pattern was found between neighboring stumps (Sbs7 × Sbs8 in site A and Sbs11–Sbs12 × Sbs14, Sbs13 × Sbs16, and Sbs15 × Sbs17 in site B). The stump-to-tree and stump-to-stump growth of the fungus rhizomorph through root contact is well known as a main mode of dispersal of *R. lignosus* in replanted fields (Bancroft 1912; Liyanage 1997; Semangun 2000).

The isolates collected from the same stumps (Sbs1 × Sbs2 and Sbs11 × Sbs12) were compatible. Some genotypes (SIGs A-1, A-5, and B-1) colonized only on single trees or stumps. All SIGs detected appeared to spread into one direction occupying an area territorially without any evidence for spatial overlapping (Fig. 2). Somatic incompatibility may restrict colonization of a single tree by multiple genotypes as consequence of competition among genotypes. Somatic incompatibility maintains individuality of secondary mycelia (Worrall 1997) and therefore prevents colonization of the same niche by genetically dissimilar mycelia.

Because our study was limited to using basidiomal isolates, it was likely that the fruited isolates had colonized neighboring trees without fructification. Therefore, the distance between compatible isolates depicted in Fig. 2 represents the minimum size estimates for the clones. Minimum size estimate for the largest genotype was 25 m in length. This kind of large clone size is similar to that occurring in some basidiomycete tree pathogens such as *Armillaria ostoyae* (Smith et al. 1994) and *A. luteobubalina* (Falk and Parbery 1995).

The occurrence of five and four clonal genotypes within a local population of *R. lignosus* in two study sites indicated that disease foci of white root were incited by different genotypes of the fungus. Genotypes belonging to distinct SIGs in a basidiomycete fungus are originated from crossings between compatible homokaryotic basidiospores (Anderson and Kohn 1995; Worrall 1997). Therefore, the presence of several SIGs in a local population of *R. lignosus* suggested that basidiospores functioned in dissemination of the fungus in the fields. Basidiospores of *R. lignosus* cannot infect living rubber trees, but can initiate infection on stumps or wood debris with subsequent spread to living tree (Liyanage 1997). The stumps or wood debris remaining in a rubber plantation are the most likely sites for production of new genotypes that originate from basidiospores. Some polymorphic DNA markers need to be used further to provide genetic estimates of the local population.

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