EFFECT OF ARBUSCULAR MYCORRHIZAL COLONIZATION ON EARLY GROWTH AND NUTRIENT CONTENT OF TWO PEAT-SWAMP FOREST TREE SPECIES SEEDLINGS, *Calophyllum hosei* AND *Ploiarium alternifolium*

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ABSTRACT

Tropical peat-swamp forests are one of the largest near-surface reserves of terrestrial organic carbon, but many peat-swamp forest tree species decreased due over-exploitation, forest fire and conversion of natural forests into agricultural lands. Among those species are slow-growing *Calophyllum hosei* and *Ploiarium alternifolium*, two species are good for construction of boats, furniture, house building and considerable attention from pharmacological viewpoint for human healthly. This study was aimed at understanding the effects of arbuscular mycorrhizal (AM) fungi on early growth of *C. hosei* and *P. alternifolium* under greenhouse condition. Seedlings of *C. hosei* and *P. alternifolium* were inoculated with AM fungi: *Glomus clarum* and *Glomus aggregatum*, or uninoculated under greenhouse condition during 6 months. AM colonization, plant growth, survival rate and nutrient content (P, Zn and B) were measured. The percentage of *C. hosei* and *P. alternifolium* ranged from 27-32% and 18-19%, respectively. Both inoculated seedling species had greater plant height, diameter, leaf number, shoot and root dry weight than control seedlings. Nutrient content of inoculated plants were increased with AM colonization. Survival rates of inoculated plants were higher (100%) than those of control plants (67%). The results suggested that inoculation of AM fungi could improve the early growth of *C. hosei* and *P. alternifolium* grown in tropical peat-swamp forest therefore this finding has greater potential impact if this innovative technology applied in field scales which are socially acceptable, commercially profitable and environmentally friendly.

Keywords: AM fungi, peat-swamp forest, *Calophyllum hosei*, *Ploiarium alternifolium*, rehabilitation

I. INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are vital components of almost all terrestrial ecosystems, forming mutually beneficial symbiosis with the roots of approximately 80% of vascular plants and frequently increasing phosphate (P) uptake and growth (Smith *et al.*, 2003). As well they increase uptake of nitrogen (N) from the soil either directly or through improvements in the plant P supply (George, 2000). One suggested that up to 80% of the P and 25% of the N taken up by a mycorrhizal fungi can be supplied by the fungus (Marschner and Dell, 1994). AM fungi can also supply other micronutrient such as Zn and Cu (Smith and Read, 1997). Besides AM fungi can play an important role on the water relation and reduce the effects of drought stress of host plants (Augé *et al.*, 2003). AM fungi activity in the soil can

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lead to increase soil aggregation (Miller and Jastrow, 2000), which improves drainage, and soil quality in sustainable maintenance of plant health (Jeffries *et al.*, 2003). In addition AM associations can reduce damage caused by soil-borne plant pathogens (Azcón-Aguilar and Barea, 1996). These associations are of great importance in forest ecology, succession, plant growth and land rehabilitation of deforested lands in the tropical rain forest (Guadarrama *et al.*, 2004). However, information about the diversity and the role of this symbiosis in Southeast Asian rain forest is limited.

Indonesia tropical peat swamp forests have the largest area of peat in the tropical zone, and are located at low altitude in the coastal and sub coastal lowlands of Irian Jaya (4.6 Mha), Sumatra (8.3 Mha) and Kalimantan (6.8 Mha) (Page *et al.*, 1999). The Dipterocarpaceae family is the dominant group of trees in Kalimantan, in abundance, density and biomass. The family of Dipterocarpaceae is considered frequently to be ectomycorrhizas (Lee, 1990). Slik *et al.* (2003) surveyed the 10 most abundant of tree families after Dipterocarpaceae were Euphorbiaceae, Myrtaceae, Sapotaceae, Lauraceae, Myristicaceae, Burseraceae, Anacardiaceae, Ebenaceae, Annonaceae and Guttiferae. These families are considered a dominance of arbuscular mycorrhizas (AM) (Tawaraya *et al.*, 2003).

The majority problem hampering the growth of tree species in peat-swamp forest is associated with the early growths of forest seedlings; the growth is often very slow and later, become stagnant. Ploiarium seeds germinate unsatisfactorily when sown immediately after collection, but Ploiarium is favoured by cuttings (Sosef *et al.*, 1998). *Calophyllum* seeds (Guttiferae) have a low germination capacity and seeds are often lost in floods during the rainy season, further limiting reproduction (Nair and Seeni, 2003). In addition, *Gonystylus bancanus* (Thymeleaceae) is commercials timbers from peat-swamp forest but this species have decreased sharply due to over exploitation and it is categorized as vulnerable in Appendix 2 CITES (CITES, 2005). Under natural condition, *G. bancanus* belongs to slow growing species and appears to have irregular flowering and fruiting habits, i.e. the month of flowering differs and it does not flower every year (Soerianegara and Lemmens, 1994).

Calophyllum and *Ploaiarium* are important species from Guttiferae family, and are widely studied by the scientific community (Khan *et al.*, 2002; Leong and Harrison, 1999). Several species of Calophyllum and Ploaiarium were used in medicine, antimicrobial activity and other biological active compound research such as coumarins, xanthones, terpenoids. In recent times *Calophyllum* species have received considerable attention from pharmacological viewpoint, since some of them produce potent inhibitors of reverse transcriptase of HIV (human immunodeficiency virus) (Reyes-Chilpa *et al.*, 1997). Some *Calophyllum* timbers are good for construction of boats and furniture and suitable for plywood, but Ploiarium is used for house building and firewood as well as charcoal for local people (Sosef *et al.*,1998).

Arbuscular mycorrhizas are the central role of microbial symbioses for the greater part of plants, under conditions of P-restriction, effect plant growth productivity, plant community development, water relations and nutrient uptake (Jeffries *et al.*, 2003). Nevertheless, very little information is available on supposedly various benefits to plant growth, nutrient uptake and survival rates conferred by the AM fungi on *Calophyllum* and *Ploaiarium* species. The present study aimed at understanding the effects of AM fungi on early growth and nutrient uptake of *Calophyllum* hosei Ridley and *Ploiarium alternifolium* (Vahl) Melchior (synonym = *Hypericum alternifolium* Vahl) (Kobuski, 1950), the slow-growing peat-swamp forest species of Indonesia, under greenhouse condition.

II. MATERIALS AND METHODS

Ultisol was collected from Haurbentes Experimental Forest, Jasinga, West Java (6° 32'-33' S, 108° 26' E) and stored in greenhouse. The soil was sieved to pass through < 5 mm and then mixed with river sand (3:1, v/v) to improve drainage. The pH (H2O) of mixed soil was 4.8 and available P (Bray-1) was 0.17 mg kg-1. The mixed soil was sterilized at 121 °C for 30 minutes. Cutting materials of *C. hosei* and *P. alternifolium* were collected from wildlings at Nyaru Menteng arboretum near Palangkaraya in Central Kalimantan, Indonesia. The cuttings were cut to a length of about 10 cm with two leaves. The leaf area was reduced to about a half of its original size. Roots of two seedling species were stimulated by rooton (Ishihara Sangyo Co.®, Japan). Crushed coconut fiber mixed with rice husks (2:1, v/v) were used for the cutting medium. The media was sterilized at 121 °C temperatures with 1 atm pressure for 50 minutes. Subsequently, the cuttings were placed in 45-hole pot-trays, one cutting was per hole. The periode growth of cuttings was 90 days. A fog evaporative cooling system was installed inside the greenhouse to lower temperature inside the propagator (Sakai *et al.*, 2002). Inoculum of AM containing *Glomus aggregatum* Schenk & Smith propagule was obtained from R&D (Research and Development) Osaka Gas Company (Japan) and *Glomus clarum* Nicholson & Schenk was isolated from peat soil of Kalampangan, Palangkaraya, Central Kalimantan by trap culture. *G. clarum* were propagated in pot cultures of *Pueraria javanica.* Plastic pots were filled with 175 g sterilized zeolite and 5 g AM inoculum. The polyethylene pots (size 15 x 10 cm) were filled with 500 g sterilized mixed soil. AM inoculation was achieved by placing 5 g AM inoculum of each species 1-3 cm under seedling. One 90-day-old *C. hosei* or *P. alternifolium* seedling was transplanted into pots. Seedlings were watered daily with tap water to a field capacity. Weed and pest controls were carried out manually. The seedlings were grown for 180 days in a greenhouse of Forest and Nature Conservation Research and Development Center (FNCRDC), Bogor, West Java.

The experiment consisted of three treatments for *C. hosei* and *P. alternifolium* seedlings (a) control; (b) *G. aggregatum*; (c) *G. clarum*. There were 15 replications of both species per treatment. Shoot height and stem diameter at 1 cm from surface of soil were measured 180 days after transplanting. Shoots were harvested and separated. Shoots and roots were oven-dried at 70° C for 72 h before weighing. Ground shoots were digested by H2SO4 and H2O2 solution (3:1, v/v). The phosphorus (P) content in the digested solution were determined by the vanado molybdate yellow method. An additional 15 seedlings of both *C. hosei* and *P. alternifolium* inoculated with *G. aggregatum, G. clarum* or uninoculated were grown as same as the seedlings in the above experiment. Numbers of alive seedling were counted 180 days after transplanting. Survival rate was calculated as follows; Survival rate (%) = number of alive seedlings / number of initial seedlings 15x 100. The roots of *C. hosei* and *P. alternifolium* were cleared in 100 g l-1 KOH for 1 h, acidified with diluted HCl and stained with 500 mg l-1 tryphan blue in lactoglycerol (Brundrett *et al.*, 1996). The percentage root length colonized by AM fungi was estimated by scoring the presence or absence of AM fungal structures (McGonigle *et al.*, 1990). Data were statistically analysed using analysis of variance (ANOVA) with the statistical software StatView 5.0 (Abacus Concepts). Comparison of means was done using the least significant difference (LSD) method at the 5% level of probability where the F-value was significant.

III. RESULTS

A. AM Colonization and Nutrient Content

G. aggregatum and *G. clarum* formed AM in roots of *C. hosei* 6 months after transplantation under greenhouse condition (Figure 1). There was no difference in percentage of colonization between *G. aggregatum* and *G. clarum*. No colonization was observed in uninoculated seedlings.

Ploiarium alternifolium roots were colonized by both AM fungal species, *G. aggregatum* and *G. clarum*, 6 months after transplantation under greenhouse condition (Figure 1). There was no difference in percentage of colonization between *G. aggregatum* and *G. clarum*. Control seedlings of *P. alternifolium* were colonized by indigenous AM fungi.

Nutrient content (P, Zn and B) were higher in shoot of *C. hosei* inoculated with *G. aggregatum* and *G. clarum* than control seedlings (Figure 1). There was no difference in shoot nutrient content between both AM fungi. AM colonization by *G. aggregatum* and *G. clarum* increased shoot *P. content* of *P. alternifolium*. There was no difference in shoot nutrient content between *G. aggregatum* and *G. clarum*.

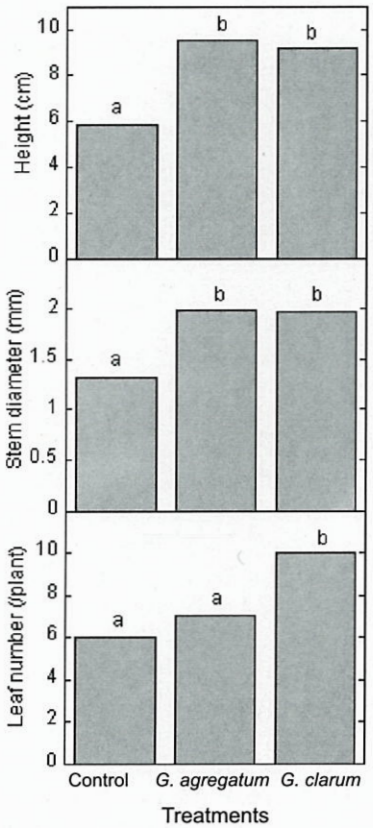

Figure 1. Plant height, diameter and leaf number of *C. hosei* inoculated with or without AM fungi, *G. aggregatum* and *G. clarum* under greenhouse condition

B. Plant Growth and Survival Rates

AM colonization of *G. aggregatum* increased plant height and stem diameter of *C. hosei* (Fig. 1). *G. aggregatum* did not increase leaf number. *G. clarum* also increased plant height, stem diameter and leaf number of *C. hosei*. Furthermore, inoculation *G. aggregatum* and *G. clarum* increased shoot dry weight and root dry weight of *C. hosei* (Figure 1). There was no difference in shoot dry weight and root dry weight of *C. hosei* between *G. aggregatum* and *G. clarum*.

AM colonization of *G. aggregatum* and *G. clarum* increased plant height, stem diameter and leaf number of *P. alternifolium* 6 months after transplantation (Figure 2). Furthermore, inoculation of *G. aggregatum* and *G. clarum* increased shoot dry weight and root dry weight (Figure 1). There was no difference in shoot height, stem diameter, leaf number, shoot and root dry weight of *P. alternifolium* between *G. aggregatum* and *G. clarum*.

AM colonization of *G. aggregatum* and *G. clarum* increased survival rates of *C. hosei* 6 months after transplantation under greenhouse conditions (Figure 3). Survival rates of P. alternifolium inoculated by both AM species were also higher than control seedlings. There was no difference in survival rates between *G. aggregatum* and *G. clarum*.

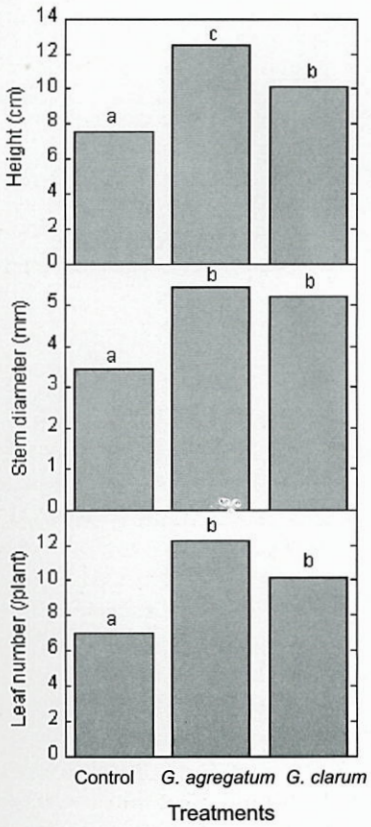

Figure 2. Plant height, diameter and leaf number of *P. alternifolium* inoculated with or without AM fungi, *G. aggregatum* and *G. clarum* under greenhouse condition

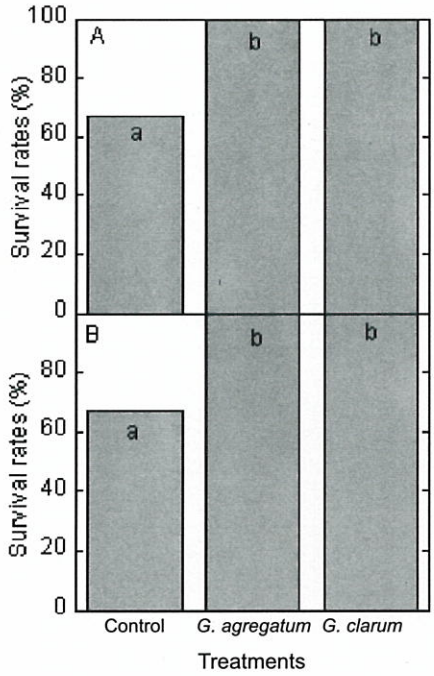

Figure 3. Survival rate of *P. alternifolium* (A) and C. hosei (B) inoculated with or without AM fungi, *G. aggregatum* and *G. clarum* under greenhouse condition

IV. DISCUSSION

G. aggregatum and *G. clarum* formed AM colonization in roots of *C. hosei* and *P. alternifolium* after 6 months transplantation under greenhouse condition (Figure 1). There were few reports of presence AM colonization in roots of plant species belonging to familiy of Guttiferae. Burslem *et al.* (1995) observed native AM colonization of *Calophyllum tetrapterum* at 27 weeks after transplantation in lowland tropical rain forest of Singapore. Tawaraya *et al.* (2003) have reported that the native AM colonization of *C. sclerophyllum* and *C. soulattri* in tropical peat-swamp forest, Central Kalimantan, Indonesia. There was no report of presence AM colonization in roots of P. alternifolium. To the best of our knowledge, this is the first observation of AM colonization in *C. hosei* and *P. alternifolium.*

Percentage of AM colonization on individual seedlings ranged from 18 to 19% for *C. hosei* and from 27 to 32% for *P. alternifolium.* (Figure 1). The level of AM colonization observed on the root of *C. hosei* and *P. alternifolium* was comparable with other reports in the literature. Tawaraya *et al.* (2003) observed the natural AM colonization of *C. sclerophyllum* and *C. soulattri* growing in peat-swamp forest as between 18 and 60%, respectively. Natural AM colonizations were observed on control *C. hosei* and *P. alternifolium* seedlings, but natural AM control seedlings (3%) were lower than *G. aggregatum* and *G. clarum.* Béreau *et al.* (2000) also

observed similar result that AM colonization of *Dicorynia guianensis* seedlings was very low in control seedlings (less than 10%). Natural AM colonization in control seedlings was highly likely originated from the soil underneath the pots since pots were placed directly above the soil surface without any border. Direct contact between the roots and the nursery floor was the possible reason of the natural mycorrhization. It was possible that wind, water and/or insects carried the AM fungi inoculum. It was likely that inoculation of both AM fungi prevented the roots from colonization by other type of fungi including the native one.

AM colonization of both seedling species by *G. aggregatum* and *G. clarum* improved shoot P, Zn and B content compared to control seedlings (Figure 1). Nutrient content was higher in shoots of inoculated (or AM fungi) seedlings than in those of controls, indicating that AM colonization improved both *C. hosei* and *P. alternifolium* nutrient uptake. This was considered as an indication of nutrient translocation in the mycelia of this fungus. Furthermore, no different between AM fungal species in this respect may indicate no different in the ability of both AM symbionts to supply P, Zn and B to the host plant, or to immobilize them within the mycelium. When these are short supply their uptake may be stimulated, and when they are present in excess their uptake may be inhibited. On soils without background contamination, improved Zn nutrition attributable to AM has been well documented in early studies (Kothari *et al.*, 1991). It is well known that the enhanced nutritional status of a plant manifests in its improved growth (Jeffries and Barea, 2001). In contrast, as mycorrhizas may be useful in nutrient or heavy metal uptake without connected enhancement of growth and P uptake (Joner and Leyval, 2001). Chen *et al.* (2003) reported that AM hyphal contribution to Zn uptake by the host plant reached its maximum value at the Zn addition level of 50 mg kg-1, in which Zn uptake via the extramatrical hyphae comprised 22% of total uptake. It is important hypothesis because Zn is an essential nutrient for plant growth and at the same time a potentially toxic metal when excessive amount are present.

Shoot P content were higher in shoots of inoculated seedlings than in those of control seedlings (Figure 1). AM fungi increased shoot P content of *Macaranga denticulate* (Euphorbiaceae) at 120 days after transplantation in Thailand (Youpensuk *et al.*, 2004). Shoot P content of *Tectona grandis* (Verbenaceae) at 60 days after transplantation from India were higher in AM seedlings (Rajan *et al.*, 2000). AM Glomus sp. and Acaulospora laevis increased P content of *Acacia concurrences* (Leguminosae) at 69 days after transplantation in Western Australia (Jasper *et al.*,1989). AM colonization increased shoot P content of *Acacia nilotica* and *Leucaena leucocephala* (Leguminosae) at 12 weeks after transplantation under greenhouse condition (Michelsen and Rosendahl, 1990). Reena and Bagyaraj (1990) confirmed that AM colonization increased P uptake of *A. nilotica* and *Calliandra calothyrsus* seedlings (Leguminosae) at 180 days after transplantation under greenhouse condtion. *G. aggregatum* and *G. clarum* increased P contents of *P. alternifolium* seedlings by up to 164-171%. In addition, both AM colonization increased P uptake of *C. hosei* by up to 39-67%. These increases were comparable to the 57% and 132 % increases that were observed in *T. grandis* (Verbenaceae) inoculated with AM fungi (Rajan *et al.*,2000).

AM fungi may improve the balance of mineral nutrition in shoot of both species, especially for trace element B (Table 1). Boron (B) is an essential micronutrient for plants but it is thought not to be essential for mycorrhizal fungi (Lehto *et al.*, 2004). The most rapid response to B deficiency is the inhibition of root elongation and reproductive growth of flowering plants (Dell and Huang, 1997). The early inhibition of root growth of seedlings,

compared to shoot growth, increases the shoot root ratio. Sakya *et al.* (2002) reported that B deficiency symptoms of *Eucalyptus globulus* seedlings appeared at day 5 in the nutrient solution containing less than 0.27 M B. Moreover, the internal critical B concentration were estimated for shoot growth between 12-16 mg B kg-1 dry weight in *E. globulus* trees up to three years of age in Southeast Asia.

Both AM fungi also increased shoot dry weight (133-152%) and root dry weight (100-186 %) of *C. hosei* (Figure 1). AM colonization of *G. aggregatum* and *G. clarum* increased shoot dry weight (106-128%) and root dry weight (170-203%) of *P. alternifolium*. Plant height, stem diameter and leaf number of AM seedlings were higher than control seedlings (Figure1 and Figure 2). Thirteen AM species increased plant height, stem diameter and leaf number of *C. calothyrsus* (Leguminosae) at 180 days after transplantation (Reena and Bagyaraj, 1990). Nine AM species increased also plant height, stem diameter and dry weight of *T. grandis* (Verbenaceae) at 180 days after transplantation. *G. clarum* colonization increased total dry weight of *Araucaria angustifolia* (Araucariaceae) at 21 months after transplantation from Brazil (Zandavalli *et al.*, 2004). AM colonization increased total dry weight of Australian rain forest tree *Flindersia brayleana* (Rutaceae) and *Acmena resa* (Myrtaceae) seedlings at 6 months after transplantation under greenhouse condition (Gehring, 2003). Total dry weight of *Dicorynia guianensis* (Caesalpiniaceae) from French Guiana at 350 days under medium light intensity was higher in AM seedlings than control seedlings (Béreau *et al.*, 2000).

In field scale, survival rate is very important because seedlings stock becomes very valuable for rehabilitating degraded forest. Survival rate of both were 100% after 6 months inoculations (Figure 3). These results were higher than survival rates of two tropical trees species from Panama inoculated by AM fungi, *Ochroma pyramidale* (97%) and *Luehe seemanii* (52%) (Kiers *et al.*, 2000). Generally, 120% seedlings stock should be prepared prior to any reforestation activity, however this study suggested that much more seedlings are needed when seedlings are not inoculated with AM fungi since field condition is much more extreme than that of nursery. On the contrary, using inoculated seedlings might prevent from unnecessary large amount of seedlings stock. These results might be significant when reforesting vast amount of areas, as it would reduce significant cost of seedlings preparation in peat-swamp area.

In this experiment, we succeeded to make rooting in two peat-swamp tree seedlings, *P. alternifolium* and *C. hosei*, with propagation cutting system under greenhouse use fogging system developed by Sakai *et al.* (2002). This technique has been used for producing seedlings of *Shorea leprosula, S. selanica* and *S. platyclados* (Dipterocarpaceae). For accelerating reforestation program, Nair and Seeni (2003) proposed that *Calophyllum* seedlings can be multiplied by tissue culture technology, but *Calophyllum* roots were growth slowly. It is promising that young rooted seedlings can acclimatized with helping AM fungi inoculation to stimulate root development and accelerate early growth of seedlings, especially for slow growing tree species origin from peat swamp forest. It is important to consider: (1) to decide and develop silviculture of mass production peat-swamp trees species technology, especially rooting seedlings development; (2) to select AM fungi for stimulating early growth seedling with different indigenous trees species under nursery condition.

V. CONCLUSION AND SUGGESTION

In conclusion, colonization of *G. aggregatum* and *G. clarum* increased plant growth, nutrient uptake and survival rates of and *C. hosei* and *P. alternifolium* seedlings 6 months after transplantation under greenhouse condition. There was no different effect between AM inoculation on plant growth with the AM fungi species used. On the other hand, *G. aggregatum* is exotic species although it can perform well with both species. When native species is unavailable than exotic AM fungi should be selected providing inoculated seedlings are improved. *G. clarum* might be used and selected if both species are selected for rehabilitating activity. Furthermore, study on the field condition is required to confirm and investigate this preliminary finding. The results suggested that AM inoculation technology can accelerate establishment of the planting stock in a large nursery scale for rehabilitating tropical peat-swamp forest.

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