Decomposition of wood, petiole, and leaf litter by *Xylaria* species from northern Thailand

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1 **Abstract**

2

3 Ten *Xylaria* isolates (five obtained from wood and five from leaf litter) collected in
4 northern Thailand were tested for their ability to decompose 13 types of wood,
5 petiole, and lamina from seven tropical tree species under pure culture conditions.
6 The mass loss of the 13 substratum types caused by the 10 *Xylaria* isolates ranged
7 from 1.2% to 37.4% of the original mass. The substratum, the origin of isolates,
8 and the contents of lignin, total carbohydrates, and nitrogen in substrata affected
9 the mass loss. Mass loss was generally in the order: petiole > lamina > wood.
10 Overall, the strains isolated from wood caused greater mass loss than the strains
11 isolated from litter. The mass loss caused by the 10 *Xylaria* isolates was
12 negatively affected by lignin and total carbohydrate contents and positively with
13 nitrogen content of the substrata. The values of mass loss in wood in the present
14 study were in the same range as those reported for other *Xylaria* isolates in
15 previous studies, whereas the mass loss in leaf litter were generally higher than
16 those of previous results, which is partly due to the relatively low lignin contents
17 in leaf litter used in the present study.

Keywords: Decomposition, Litter, Tropical forest, *Xylaria*

Introduction

Fungi in the genus *Xylaria* (Xylariaceae, Xylariales, Sordariomycetes, Ascomycota) are major components of mycobiota in tropical forests (Whalley 1993, 1996, 1997; Rogers 2000) and function as decomposers (e.g., Rogers et al. 1987, 1988; Gonzalez and Rogers 1989; Van der Gucht 1996; Osono 2007; Osono et al. 2008, 2009), endophytes (Rodrigues et al. 1993; Læssøe and Lodge 1994; Rodrigues 1994; Bayman et al. 1998; Okane et al. 2008), pathogens (Ko and Kunimoto 1991), and symbionts with termite nests (Rogers et al. 2005; Ju and Hsieh 2007; Okane and Nakagiri 2007; Visser et al. 2009). Saprobic *Xylaria* species inhabit the wood, petiole and lamina of tropical trees and take part in decomposition and mineralization of these plant litters. Previous pure culture studies have demonstrated that tropical wood-inhabiting *Xylaria* isolates are able to cause significant mass loss in wood blocks of temperate beech and pine (Pointing et al. 2003). Tropical endophytic *Xylaria* species have also been shown to

produce extracellular enzymes to decompose plant substrates (Rodrigues et al. 1993; Pointing et al. 2003, 2005). However, few data have been available regarding the ability of tropical *Xylaria* species to cause mass loss in wood and leaf litter of tropical trees. Such studies are crucial for the understanding of functional roles of *Xylaria* species in decomposition processes in tropical forests.

The purposes of the present study were to examine the ability of 10 *Xylaria* isolates (five obtained from wood and five from leaf litter) to decompose wood, petiole, and lamina of seven tropical tree species in pure culture tests and to compare these results with the decomposing ability of other *Xylaria* isolates reported in previous studies. The *Xylaria* isolates were collected in northern Thailand and tested for their ability to cause loss of mass of 13 substratum types (four woods, three petioles, and six laminas) during the laboratory incubation. We hypothesized that wood and litter isolates would be able to decompose both wood, petioles, and lamina regardless of the isolate origin and that petiole and lamina would decompose faster than wood, which was attributable to chemical quality of the substrata.

Materials and methods

Source of fungi and substrata for decomposition tests

Ten *Xylaria* isolates were used in the test, including five from wood and five from leaf litter (Table 1). The 10 isolates were collected in October and November 2004 at three study sites in northern Thailand: Queen Sirikit Botanic Garden and Doi Suthep National Park in Chiang Mai, and Naresuan University, Phayao Campus, in Phayao. Isolates TP5BS72 and TP5BS101 were isolated from decomposing *Shorea obtusa* leaves by means of the surface disinfection method (Osono et al. 2009). The other eight isolates were obtained from mass ascospores discharged from fruiting bodies collected from twigs, branches, or logs, or petioles or primary veins of tree leaves. Isolates were maintained on slants of 1% malt extract agar medium [malt extract 1% and agar 2% (w/v)] at 20˚C in darkness until the tests were performed.

Phylogenetic placement of the isolates was determined based on morphological observations and on the DNA sequence of amplicons (566 to 596 bp)

of rDNA ITS region obtained using primers ITS5 and ITS4 (White et al. 1990), basically according to the method described in Hirose and Osono (2006). The sequences of *Xylaria* isolates were compared with those of known species using BLAST searching against the Genbank database (Table 1, Fig. 1). Two isolates (TC041101 and TC041107) were identified as *X. polymorpha* and *X. grammica* with reference to morphological observations of fruiting bodies from which the isolates were obtained, whereas we were unable to identify the other six isolates from fruiting bodies to species. In the present study, we referred to *Xylaria* isolates obtained from fruiting bodies on woody substrata (twigs, branches, and logs) as wood isolates and those from leaf litter (laminae, petioles, and vein) as litter isolates. The terms wood and litter isolates do not indicate that these isolates are representatives of *Xylaria* species inhabiting or fruiting on wood and litter. In fact, molecular phylogenetic analysis showed that TP041001 from twig and TP5BS101 isolated from lamina can be regarded as being identical with respect to their base sequences of ITS region (Table 1), suggesting that the distinction between the wood and litter isolates is tentative.

Wood, petioles, and laminae of seven tropical tree species were collected

in the three study sites in February, October, and November 2004 and used as substrata in the pure culture test (Table 2). Wood blocks (approx. 10 x 10 x 5 mm) were cut out from living trees of *Dipterocarpus tuberculatus*, *Tectona grandis*, *Quercus kingiana*, and *Shorea obtusa*. Newly shed leaves of *Bauhinia variegata*, *Macalanga denticulata*, *D. tuberculatus*, *T. grandis*, *S. obtusa*, and *Pinus kesiya* without obvious fungal or faunal attack were collected from the forest floor of the three study sites. The leaves were separated into petiole and lamina for *B. variegata*, *M. denticulata*, and *D. tuberculatus*. Lamina was cut into strips 1 cm wide (broadleaved trees) or pieces 2 cm in length (*Pinus* needles). The wood, petiole, and lamina were oven-dried at 40˚C for 1 week and preserved in vinyl bags until the experiment was started. Trees are referred to as their genus names in the present study for the sake of simplicity.

Decomposition tests

Samples of wood, petiole, and lamina were weighed and sterilized by exposure to ethylene oxide gas at 60˚C for 6 hours. The sterilized materials (approx. 400 mg

wood, approx. 400 mg petiole, and 300 mg lamina per dish) were placed on the surface of Petri dishes (9 cm diameter) containing 20 ml of 2% agar medium. Inocula for each assessment were cut out of the margin of previously inoculated Petri dishes on 1% malt extract agar medium with a sterile cork borer (6 mm diameter) and placed on the agar medium adjacent to materials, one plug per plate. The plates were incubated for 12 weeks at 20˚C in the dark. The plates were sealed firmly with laboratory film during incubation so that moisture did not limit decomposition on the agar medium. After incubation, the plant materials were retrieved, oven-dried at 40˚C for 1 week, and weighed. The initial, undecomposed materials were also sterilized, oven-dried again at 40˚C for 1 week, and weighed to determine the original mass. Four plates were prepared for each isolate and each substratum (wood, petiole, or lamina), and four uninoculated plates served as a control for each substratum. Mass loss of wood, petiole, and lamina was determined as a percentage of the original mass, taking the mass loss of materials in the uninoculated and incubated control treatment into consideration, and the mean values were calculated for each isolate and each substratum. Prior to the tests, the sterilized substrata were placed on 1% malt extracted agar medium, and

after 8 weeks of incubation at 20˚C in darkness, no microbial colonies had developed on the plates. Thus, the effectiveness of the sterilization method used in the present study was assured.

Chemical analysis

The initial, undecomposed materials were combined to make one sample for each substratum and ground in a laboratory mill (0.5 mm screen). The amount of lignin in the samples was estimated by means of gravimetry, using hot sulfuric acid digestion (King and Heath 1967). Samples were extracted with alcohol-benzene at room temperature (15-20˚C), and the residue was treated with 72% sulfuric acid (v/v) for 2 h at room temperature with occasional stirring. The mixture was diluted with distilled water to make a 2.5% sulfuric acid solution and autoclaved at 120˚C for 60 min. After cooling, the residue was filtered and washed with water through a porous crucible (G4), dried at 105˚C, and weighed as acid-insoluble residue. The filtrate (autoclaved sulfuric acid solution) was used for total carbohydrate analysis. The amount of carbohydrate in the filtrate was estimated

by means of the phenol-sulfuric acid method (Dubois *et al.* 1956). One ml of 5% phenol (v/v) and 5 ml of 98% sulfuric acid (v/v) were added to the filtrate. The optical density of the solution was measured using a spectrophotometer at 490 nm, using known concentrations of D-glucose as standards. Total N concentration was measured using a combustion method with an automatic gas chromatograph (NC analyzer SUMIGRAPH NC-900, Sumitomo Chemical, Osaka, Japan).

Statistical analysis

Pearson's correlation coefficients were calculated among initial chemical properties. Factors affecting the mean values of mass loss of each substratum for individual fungal isolates were analyzed with two-way ANOVA with substratum (wood, petiole, lamina), isolate origin (wood, litter), and the interactions of substratum × isolate origin as independent variables. We included this interaction to test the substratum specificity of *Xylaria* isolates of wood and litter origin. Regression analyses were performed for linear relationships between mass loss values and initial chemical properties. Mass loss values were arcsin-transformed

because the data were in the form of proportion. JMP version 6.0 for Macintosh was used for the statistical analyses.

Results

Initial chemical composition

The 13 types of wood, petiole, and lamina examined in the pure culture tests varied in their initial chemical composition (Table 2). The lignin content in wood and lamina was generally greater than that in petiole. The total carbohydrate content was greater in wood than in petiole and lamina. The content of extractives was highest in lamina, followed by wood and then by petiole. Nitrogen content was generally in the order: lamina > petiole > wood. When all substratum types were included (n=13), total carbohydrate content was significantly and negatively correlated with the content of extractives ($R=-0.629$, $P<0.05$) and nitrogen ($R=-0.658$, $P<0.05$).

1 Mass loss

2

3 The mass loss of the 13 types of wood, petiole, and lamina caused by the 10
4 *Xylaria* isolates tested in the pure culture tests ranged from 1.2% to 37.4% (Table
5 3). The mean greatest mass losses were observed for *Bauhinia* petiole and lamina
6 and *Tectona* lamina, whereas the mean lowest mass losses were observed for
7 *Tectona* wood and *Dipterocarpus* lamina (Table 3). The mean greatest mass loss of
8 wood, petiole, and lamina was caused by TP5BS101, followed by TP041004 and
9 TP041001 (Table 3).

10

11 Factors affecting mass loss

12

13 Substratum and isolate origin were significantly affected the mass loss
14 (substratum, df=2, F=14.81, P<0.001; isolate origin, df=1, F=5.62, P=0.019). Mass
15 loss tended to be in the order: wood < lamina < petiole, and wood isolates in
16 general caused greater mass loss than litter isolates (Fig. 2). The substratum ×
17 isolate origin interaction was not significant (df=2, F=0.81, P=0.84), suggesting

that the substratum specificity was not significant for the 10 *Xylaria* isolates. The mass loss was significantly and negatively correlated with lignin content (n=130, R^2=0.14, F=20.3, P<0.0001) and total carbohydrate content (n=130, R^2=0.06, F=8.7, P=0.0038) and significantly and positively with nitrogen content (n=130, R^2=0.09, F=13.2, P=0.004) (Fig. 2). Mass loss was not significantly correlated with the content of extractives (n=130, R^2=0.01, F=1.67, P=0.20).

Discussion

The present study showed that the 10 *Xylaria* isolates from northern Thailand examined here were capable of causing mass loss of wood, petiole, and lamina of tropical tree species, except that the litter isolate TC041105 was incapable of causing significant mass loss of wood (Table 3). Both wood and litter isolates decomposed wood, petiole, and lamina regardless of the original substrata (i.e. wood or litter), as was demonstrated by the non-significant effect of the substratum × isolate origin interaction. Such decomposition has been attributed to the ability of *Xylaria* to produce cellulolytic and ligninolytic enzymes

responsible for the hydrolysis, solubilization, and/or mineralization of organic compounds (Whalley 1996; Rogers 2000; Pointing et al. 2003). Here, the results are discussed with reference to previous results regarding wood and litter decomposition by other *Xylaria* isolates in pure culture conditions (reviewed in Table 4).

The mass loss values of wood reported in the present study are in the same range as those reported to be caused by other *Xylaria* isolates from temperate wood, despite the differences in experimental methods among the previous studies (Table 4). In contrast, the mass loss values for tropical leaf litter in the present study were generally higher than those caused by other *Xylaria* isolates for other temperate and tropical leaf litter examined with the same experimental methods (Table 4). In fact, the highest values in the present study were recorded for leaf litter of *Bauhinia*, *Tectona*, and *Macalanga,* which had relatively low lignin (188-264 mg/g) and high nitrogen contents (9.2-14.2 mg/g) (Table 2), whereas the lignin content of substrata used in the previous studies was 212-454 mg/g, and the N content 6.0-11.5 mg/g. Therefore, the relatively higher values of mass loss in the present study were partly attributable to the difference

in the chemical quality of the leaf litter.

The substrata (wood, petiole, lamina), the isolate origin, and chemical composition of the substrata affected the mass loss (Fig. 2). The lower mass loss of wood was primarily attributable to higher lignin and lower nitrogen contents (Table 2). Lignin is one of the most recalcitrant components in wood and leaf litter and often retards fungal growth and decomposition, and nitrogen is a major essential element limiting fungal growth and enzyme production (Eriksson et al. 1990; Sinsabaugh et al. 2002; Waldrop and Zak 2006; Boberg et al. 2008). Alternatively, factors potentially influencing the fungal decomposition of wood and leaf components could include the anatomical structures of tissues and secondary plant metabolites that inhibit or stimulate fungal growth, such as essential oils and heartwood components (Lindeberg et al. 1980; Alfenas et al. 1982). Few studies other than the present one have compared the decomposition of wood and leaf components by single *Xylaria* isolates (see Table 4).

The wood isolates tended to cause greater mass loss than the litter isolates (Table 3), consistent with the finding of Osono and Takeda (2002) that *Xylaria* isolates from woody cupules caused slightly greater mass loss than those

from leaf litter. No other studies have made similar comparisons (Table 4), making it difficult to draw general conclusions regarding the difference in potential decomposing capabilities of wood- and litter-inhabiting *Xylaria*. It should be noted that *Xylaria* sp. TP041001 (fruiting on a twig) was genetically identical to TP5BS101 (which was isolated from leaf lamina collected in the same study site as TP041001). This indicates that some species are able to colonize both wood and leaf substrata, and makes the distinction between 'wood-' and 'litter-inhabiting *Xylaria* species' less clear. Further studies will be necessary to compare decomposing and enzymatic activities of between *Xylaria* species representative of wood and litter decomposers.

We can address two more suggestions from Table 4 regarding the abilities of tropical *Xylaria* species to decompose plant substrata. First, the 10 *Xylaria* isolates caused lower mass loss values for *Pinus* needles than for broadleaved lamina (Table 3), consistent with the patterns found in previous studies showing that *Xylaria* isolates caused lower mass loss for coniferous wood or leaf litter than for broadleaved litter (Table 4). This can be explained by the facts that coniferous substrata generally have higher lignin content than broadleaves ones (e.g., 379

mg/g in *Pinus kesiya* needles vs 188-340 mg/g in broadleaved lamina, Table 2) and that conifers contain primarily guaiacyl lignin, which is more resistant to fungal decomposition than syringyl lignin in angiosperms (Eriksson et al. 1990). Secondary, few studies but the present one have examined the decomposition of tropical wood by *Xylaria* isolates (see Table 4). *Xylaria* species are well represented in the tropics, and tropical *Xylaria* species are expected to be diverse in terms of species richness, functioning, and interactions with plant hosts (Whalley 1993, Rogers 2000). Future studies should expand the investigation to more diverse *Xylaria* species of wood and litter origin and their abilities to decompose tropical substrata, including coniferous tissues.

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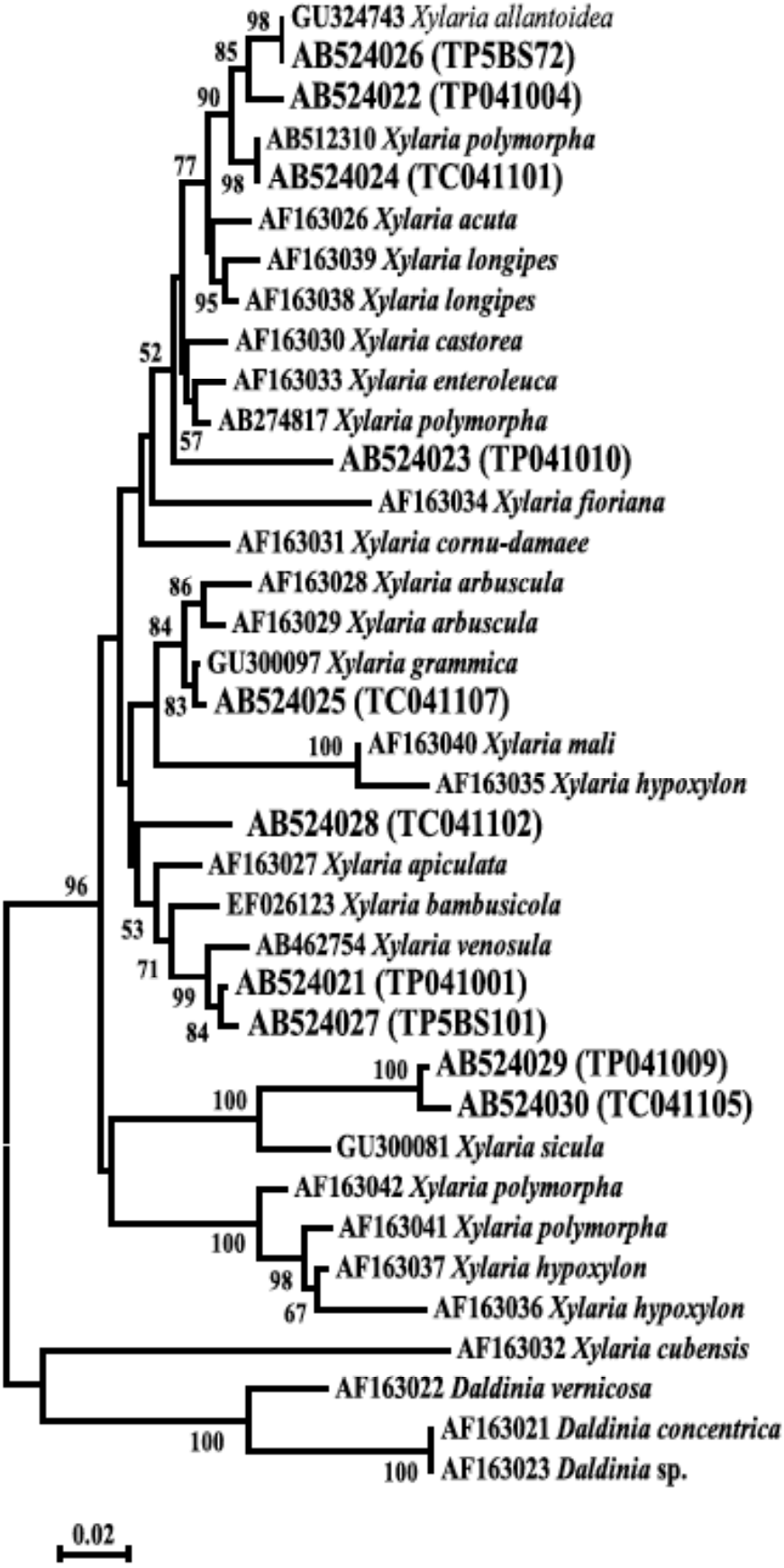
Figure legend

Fig 1 - Neighbour-joining tree based on the rDNA ITS sequences of the *Xylaria* species used in the present study. Twenty sequences used in Lee et al. (2000) and best identified BLAST match taxa were also included in the phylogenetic analysis. Bootstrap values above 50% are given adjacent to the corresponding node.

Fig 2 - Mean values of mass loss for wood, petiole, and lamina (upper, left) and wood and litter isolates (upper, right) and relationship between the mean value of mass loss for 10 *Xylaria* isolates and the initial contents of lignin (middle, left), total carbohydrate (middle right), and nitrogen (lower, left). Open squares, wood; open circles, petiole; open triangles, lamina.



1 Osono et al. Fig 1

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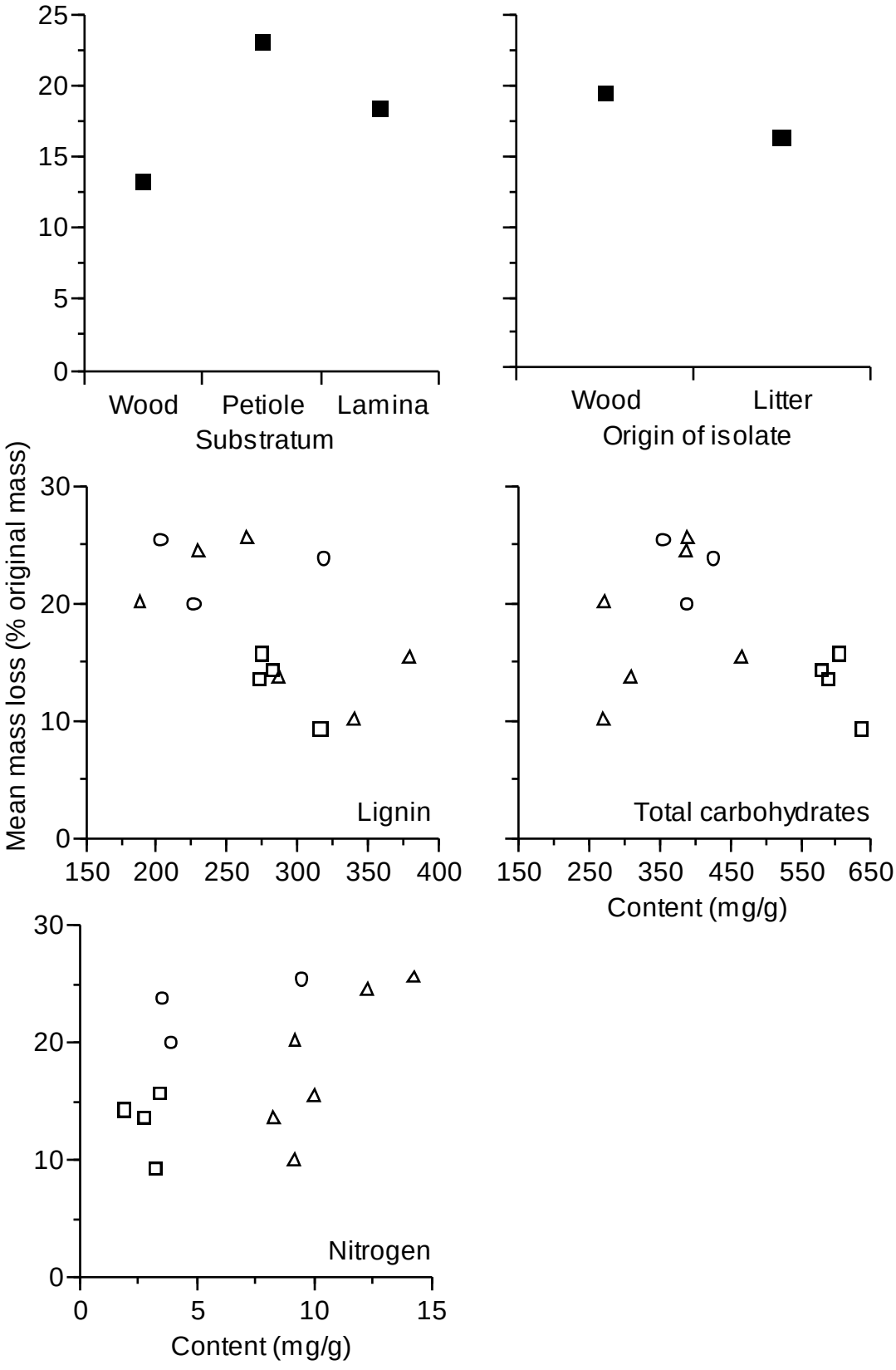
1 Osono et al. Fig 2



Osono et al. Table 1

Table 1 - *Xylaria* isolates used in the test.

Strain code	Collection site	Month of 2004	Substratum	Method of isolation	DDBJ accession number	Best identified BLAST match taxa (accession number)	Sequence similarity %
Wood isolates							
TP041001	NU	Oct	Twig	MA	AB524021	*Xylaria venosula* (AB462754)	96
TP041004	NU	Oct	Branch	MA	AB524022	*Xylaria allantoidea* (GU324743)	97
TP041010	NU	Oct	Branch	MA	AB524023	*Xylaria polymorpha* (AB274817)	93
TC041101	QSBG	Nov	Log	MA	AB524024	*Xylaria polymorpha* (AB512310)	99
TC041107	DS	Nov	Log	MA	AB524025	*Xylaria grammica* (GU300097)	98
Litter isolates							
TP5BS72	NU	Oct	Lamina	SD	AB524026	*Xylaria allantoidea* (GU324743)	99
TP5BS101	NU	Oct	Lamina	SD	AB524027	*Xylaria venosula* (AB462754)	96
TC041102	QSBG	Nov	Petiole	MA	AB524028	*Xylaria bambusicola* (EF026123)	93
TP041009	NU	Oct	Petiole	MA	AB524029	*Xylaria sicula* (GU300081)	91
TC041105	DS	Nov	Vein	MA	AB524030	*Xylaria sicula* (GU300081)	91

NU Naresuan University, Phayao Campus, Phayao; QSBG Queen Sirikit Botanic Garden, Chiang Mai; DS Doi Suthep, Chiang Mai.

MA mass ascospores from fruiting body, SD surface disinfection of lamina.

Osono et al. Table 2

Table 2 - Contents (mg/g) of organic chemical components and nitrogen, in wood, petiole, and lamina of seven tropical tree species used in pure culture decomposition tests.

Type	Lignin	Total carbohydrate	Extractives	Nitrogen
Wood				
Shorea	275	606	25	3.4
Quercus	274	589	22	2.8
Dipterocarpus	282	581	32	2.0
Tectona	316	636	39	3.3
Mean	*287*	*603*	*29*	*2.9*
Petiole				
Bauhinia	203	356	4	9.5
Macalanga	227	389	12	3.9
Dipterocarpus	318	426	39	3.5
Mean	*249*	*390*	*18*	*5.6*
Lamina				
Bauhinia	229	387	45	12.2
Tectona	264	390	63	14.2
Macalanga	188	271	165	9.2
Pinus	379	467	56	10.0
Shorea	287	310	103	8.3
Dipterocarpus	340	270	121	9.2
Mean	*281*	*349*	*92*	*10.5*

1 Osono et al. Table 3

2

3 Table 3 - Mass loss (% original mass) of wood, petiole, and lamina decomposed at 20˚C by 10 *Xylaria* isolates in vitro. Values
4 indicate mean ± standard errors (n=4).

	Wood isolates					Litter isolates				
	TP041001	TP041004	TP041010	TC041101	TC041107	TP5BS72	TP5BS101	TC041102	TP041009	TC041105
Wood										
Shorea	18.1±1.7	28.4±2.2	21.8±0.7	21.2±0.3	8.8±0.3	18.8±1.9	20.7±3.1	8.8±0.7	7.8±0.8	3.1±0.6
Quercus	19.6±5.4	17.7±0.3	13.3±1.9	16.2±2.4	9.0±1.3	14.3±0.5	18.4±0.7	16.5±1.0	9.8±0.4	1.2±0.4
Dipterocarpus	18.3±1.1	17.4±0.6	13.7±0.5	16.4±1.1	10.2±0.3	14.1±1.0	25.9±0.9	13.9±1.8	8.6±0.4	4.3±0.5
Tectona	11.8±3.3	11.3±1.0	12.2±0.7	4.8±0.7	8.5±0.3	10.3±1.9	19.4±1.3	8.0±0.7	4.5±0.4	2.4±0.9
Petiole										
Bauhinia	34.3±3.8	32.6±2.9	37.4±0.3	20.9±1.0	23.1±1.3	22.4±1.8	26.1±2.3	23.8±2.1	22.2±1.0	11.3±1.4
Macalanga	23.0±2.4	19.2±3.3	31.5±2.1	15.8±1.6	12.9±0.9	12.7±3.5	24.0±3.4	17.7±1.1	26.9±2.9	16.2±4.4
Dipterocarpus	30.7±1.6	25.3±6.8	28.2±3.5	17.2±0.4	13.8±1.1	29.6±1.6	28.7±3.6	24.6±3.5	23.3±3.8	16.5±1.7
Lamina										
Bauhinia	29.8±1.3	28.1±1.8	32.6±0.8	28.1±0.6	23.0±0.7	23.6±2.5	34.0±1.1	24.9±1.5	12.0±0.2	10.3±1.0
Tectona	31.8±3.1	24.0±2.9	36.2±1.8	22.4±1.9	23.9±1.0	29.6±1.9	34.3±1.2	27.7±2.1	17.2±0.7	10.5±0.2
Macalanga	23.2±3.8	25.9±1.8	12.4±2.1	25.8±0.3	25.5±2.1	27.8±1.4	26.8±1.4	9.0±2.6	15.4±1.1	11.3±1.4
Pinus	15.7±2.3	22.6±0.8	15.0±1.3	13.8±0.7	16.0±0.7	22.1±0.4	25.8±1.4	5.8±0.4	10.4±0.9	8.4±0.5
Shorea	16.7±3.0	21.3±1.2	10.4±2.4	10.0±1.8	14.0±1.9	17.9±2.9	23.7±3.4	7.5±0.4	9.8±2.5	6.7±1.2
Dipterocarpus	12.7±2.0	14.6±1.3	6.9±2.0	4.1±0.5	11.8±1.7	13.9±1.4	20.0±1.3	4.6±0.6	7.8±0.5	5.3±0.7

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2

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Table 4 - A review of mass loss (% original mass) of wood and litter of tropical and temperate trees caused by wood- and litter-isolates of *Xylaria* of tropical and temperate origins. **Bold case** indicates tropical isolates of *Xylaria*. *Italicized numbers* indicate the data of conifer decomposition. Numbers of isolates tested are in parentheses.

	Wood decomposition		Litter decomposition		Method				Reference
Substrata	Wood isolates	Litter isolates	Wood isolates	Litter isolates	Medium	Sterilization	Temperature (˚C)	Period (wk)	
Temperate trees									
Fagus sylvatica	**2.6-10.3 (12)**	nd	nd	nd	S	AC	25	12	1
Pinus sylvestris	***0.0-3.4 (12)***	nd	nd	nd	S	AC	25	12	
Betula verrucosa	14.9-40.6 (4)	nd	nd	nd	S	AC	25	16	2
Pinus sylvestris	*3.3-18.4 (4)*	nd	nd	nd	S	AC	25	16	
Betula alleghaniensis	5.9-6.6 (2)	nd	nd	nd	S	AC	25	12	3
Pinus taeda	*2.9-5.2 (2)*	nd	nd	nd	S	AC	25	12	
Fagus crenata	2.7-5.4 (3)	nd	nd	nd	M	EG	25	12	4
Fagus crenata	nd	nd	4.0-14.4 (2)*	5.5-9.9 (8)	W	AC	20	8	5
Camellia japonica	nd	nd	nd	13.1-18.1 (3)	W	EG	20	12	6
Larix leptolepis	nd	nd	nd	*4.5 (1)*	W	EG	20	12	7
Chamaecyparis obtusa	nd	nd	nd	*3.0 (1)*	W	EG	20	12	8
Tropical trees									

Castanopsis sieboldii	nd	nd	**13.5 (1)**	**13.8 (1)**	W	EG	20	12	9
Shorea obtusa	nd	nd	nd	**9.3-10.3 (2)**	W	EG	20	12	10
Broadleaved, 4 to 5 tree sp.	**4.8-28.4 (5)**	**1.2-25.9 (5)**	**4.1-36.2 (5)**	**4.6-34.3 (5)**	W	EG	20	12	11
Coniferous, 1 tree sp.	nd	nd	***13.8-22.6 (5)***	***5.8-25.8 (5)***	W	EG	20	12	

* *Xylaria carpophila* inhabiting cupules.

nd not determined.

Medium: S soil, M, malt extract agar, W water agar.

Sterilization of substrata: AC autoclaving, EG ethylene oxide gas.

Reference: 1 Pointing et al. (2003), 2 Nilsson et al. (1989), 3 Worrall et al. (1997), 4 Fukasawa et al. (2005), 5 Osono and Takeda (2002), 6 Koide et al. (2005), 7 Osono et al. (2003), 8 Osono et al. (2006), 9 Osono et al. (2008), 10 Osono et al. (2009), 11 This study.