

Title	Decomposition of wood, petiole and leaf litter by Xylaria species from northern Thailand
Author(s)	Osono, Takashi; To-Anun, Chaiwat; Hagiwara, Yusuke; Hirose, Dai
Citation	Fungal Ecology (2011), 4(3): 210-218
Issue Date	2011-06
URL	http://hdl.handle.net/2433/158360
Right	© 2010 Elsevier Ltd and The British Mycological Society.
Type	Journal Article
Textversion	author

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

3

4 Takashi Osono^{*,1}, Chaiwat To-Anun², Yusuke Hagiwara³, Dai Hirose⁴

5

6 ¹ Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113 Japan

7 ² Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200 Thailand

8 ³ Graduate School of Agriculture, Kyoto University, Kyoto 606-8502 Japan

9 ⁴ College of Pharmacy, Nihon University, Funabashi, Chiba 274-8555 Japan

10

11 * Corresponding author. Tel.: +81 77 549 8252; fax: +81 77 549 8201.

12 E-mail address: tosono@ecology.kyoto-u.ac.jp

13 URL: <http://www.ecology.kyoto-u.ac.jp/~tosono/>

14

15

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.

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

2

3 **Introduction**

4

5 Fungi in the genus *Xylaria* (Xylariaceae, Xylariales, Sordariomycetes,
6 Ascomycota) are major components of mycobiota in tropical forests (Whalley 1993,
7 1996, 1997; Rogers 2000) and function as decomposers (e.g., Rogers et al. 1987,
8 1988; Gonzalez and Rogers 1989; Van der Gucht 1996; Osono 2007; Osono et al.
9 2008, 2009), endophytes (Rodrigues et al. 1993; Læssøe and Lodge 1994;
10 Rodrigues 1994; Bayman et al. 1998; Okane et al. 2008), pathogens (Ko and
11 Kunimoto 1991), and symbionts with termite nests (Rogers et al. 2005; Ju and
12 Hsieh 2007; Okane and Nakagiri 2007; Visser et al. 2009). Saprobic *Xylaria*
13 species inhabit the wood, petiole and lamina of tropical trees and take part in
14 decomposition and mineralization of these plant litters. Previous pure culture
15 studies have demonstrated that tropical wood-inhabiting *Xylaria* isolates are able
16 to cause significant mass loss in wood blocks of temperate beech and pine
17 (Pointing et al. 2003). Tropical endophytic *Xylaria* species have also been shown to

1 produce extracellular enzymes to decompose plant substrates (Rodrigues et al.
2 1993; Pointing et al. 2003, 2005). However, few data have been available
3 regarding the ability of tropical *Xylaria* species to cause mass loss in wood and
4 leaf litter of tropical trees. Such studies are crucial for the understanding of
5 functional roles of *Xylaria* species in decomposition processes in tropical forests.

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

17

1 **Materials and methods**

2

3 Source of fungi and substrata for decomposition tests

4

5 Ten *Xylaria* isolates were used in the test, including five from wood and five from
6 leaf litter (Table 1). The 10 isolates were collected in October and November 2004
7 at three study sites in northern Thailand: Queen Sirikit Botanic Garden and Doi
8 Suthep National Park in Chiang Mai, and Naresuan University, Phayao Campus,
9 in Phayao. Isolates TP5BS72 and TP5BS101 were isolated from decomposing
10 *Shorea obtusa* leaves by means of the surface disinfection method (Osono et al.
11 2009). The other eight isolates were obtained from mass ascospores discharged
12 from fruiting bodies collected from twigs, branches, or logs, or petioles or primary
13 veins of tree leaves. Isolates were maintained on slants of 1% malt extract agar
14 medium [malt extract 1% and agar 2% (w/v)] at 20°C in darkness until the tests
15 were performed.

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

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

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

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

13

14 Decomposition tests

15

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

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

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

4

5 Chemical analysis

6

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

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

7

8 Statistical analysis

9

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

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

3

4 **Results**

5

6 Initial chemical composition

7

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

17

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 \times
17 isolate origin interaction was not significant ($df=2$, $F=0.81$, $P=0.84$), suggesting

1 that the substratum specificity was not significant for the 10 *Xylaria* isolates. The
2 mass loss was significantly and negatively correlated with lignin content (n=130,
3 $R^2=0.14$, $F=20.3$, $P<0.0001$) and total carbohydrate content (n=130, $R^2=0.06$,
4 $F=8.7$, $P=0.0038$) and significantly and positively with nitrogen content (n=130,
5 $R^2=0.09$, $F=13.2$, $P=0.004$) (Fig. 2). Mass loss was not significantly correlated with
6 the content of extractives (n=130, $R^2=0.01$, $F=1.67$, $P=0.20$).

7

8 **Discussion**

9

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

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

6 The mass loss values of wood reported in the present study are in the
7 same range as those reported to be caused by other *Xylaria* isolates from
8 temperate wood, despite the differences in experimental methods among the
9 previous studies (Table 4). In contrast, the mass loss values for tropical leaf litter
10 in the present study were generally higher than those caused by other *Xylaria*
11 isolates for other temperate and tropical leaf litter examined with the same
12 experimental methods (Table 4). In fact, the highest values in the present study
13 were recorded for leaf litter of *Bauhinia*, *Tectona*, and *Macalanga*, which had
14 relatively low lignin (188-264 mg/g) and high nitrogen contents (9.2-14.2 mg/g)
15 (Table 2), whereas the lignin content of substrata used in the previous studies was
16 212-454 mg/g, and the N content 6.0-11.5 mg/g. Therefore, the relatively higher
17 values of mass loss in the present study were partly attributable to the difference

1 in the chemical quality of the leaf litter.

2 The substrata (wood, petiole, lamina), the isolate origin, and chemical
3 composition of the substrata affected the mass loss (Fig. 2). The lower mass loss of
4 wood was primarily attributable to higher lignin and lower nitrogen contents
5 (Table 2). Lignin is one of the most recalcitrant components in wood and leaf litter
6 and often retards fungal growth and decomposition, and nitrogen is a major
7 essential element limiting fungal growth and enzyme production (Eriksson et al.
8 1990; Sinsabaugh et al. 2002; Waldrop and Zak 2006; Boberg et al. 2008).
9 Alternatively, factors potentially influencing the fungal decomposition of wood
10 and leaf components could include the anatomical structures of tissues and
11 secondary plant metabolites that inhibit or stimulate fungal growth, such as
12 essential oils and heartwood components (Lindeberg et al. 1980; Alfenas et al.
13 1982). Few studies other than the present one have compared the decomposition
14 of wood and leaf components by single *Xylaria* isolates (see Table 4).

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

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

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

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

11

12 **Acknowledgments**

13

14 We thank Dr. M. Kakishima, Dr. S. Tokumasu, Dr. H. Takeda, Dr. S. Khamyong,
15 and Mr. T. Seramethakun for help with fieldwork in Thailand; Dr. A. Mori for help
16 with statistical analysis; Mr. O. Tateno for help with molecular analysis of *Xylaria*
17 isolates; and Dr. Elizabeth Nakajima for critical reading of the manuscript. This

1 study was supported by Global COE Program A06 to Kyoto University.

2

3 **References**

4

5 Alfenas AC, Hubbes M, Couto L, 1982. Effect of phenolic compounds from

6 *Eucalyptus* on the mycelial growth and conidial germination of

7 *Cryphonectria cubensis*. Canadian Journal of Botany **60**: 2535-2541.

8 Bayman P, Angulo-Sandoval P, Báez-Ortiz Z, Lodge DJ, 1998. Distribution and

9 dispersal of *Xylaria* endophytes in two tree species in Puerto Rico.

10 Mycological Research **102**: 944-948.

11 Boberg J, Finlay RD, Stenlid J, Näsholm T, Lindahl BD, 2008. Glucose and

12 ammonium additions affect needle decomposition and carbon allocation by

13 the litter degrading fungus *Mycena epipterygia*. Soil Biology &

14 Biochemistry **40**: 995-999.

15 Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F, 1956. Colorimetric

16 method for determination of sugars and related substances. Analytical

17 Chemistry **28**: 350-356.

- 1 Eriksson KEL, Blanchette RA, Ander P, 1990. Microbial and enzymatic
2 degradation of wood and wood components. Springer, Berlin.
- 3 Fukasawa Y, Osono T, Takeda H, 2005. Decomposition of Japanese beech wood by
4 diverse fungi isolated from a cool temperate deciduous forest. *Mycoscience*
5 **46**: 97-101.
- 6 Gonzalez FSM, Rogers JD, 1989. A preliminary account of *Xylaria* of Mexico.
7 *Mycotaxon* **34**: 283-373.
- 8 Hirose D, Osono T, 2006. Development and seasonal variations of *Lophodermium*
9 populations on *Pinus thunbergii* needle litter. *Mycoscience* **47**: 242-247.
- 10 Ju YM, Hsieh HM, 2007. *Xylaria* species associated with nests of *Odontotermes*
11 *formosanus* in Taiwan. *Mycologia* **99**: 936-957.
- 12 King HGC, Heath GW, 1967. The chemical analysis of small samples of leaf
13 material and the relationship between the disappearance and composition
14 of leaves. *Pedobiologia* **7**: 192-197.
- 15 Ko WH, Kunimoto RK, 1991. Quick decline of macadamia trees: association with
16 *Xylaria arbuscula*. *Plant Pathology* **40**: 643-644.
- 17 Koide K, Osono T, Takeda H, 2005. Fungal succession and decomposition of

- 1 *Camellia japonica* leaf litter. *Ecological Research* **20**: 599-609.
- 2 Læssøe T, Lodge DJ, 1994. Three host-specific *Xylaria* species. *Mycologia* **86**:
3 436-446.
- 4 Lee JS, Ko KS, Jung HS, 2000. Phylogenetic analysis of *Xylaria* based on nuclear
5 ribosomal ITS-5.8S-ITS2 sequences. *FEMS Microbiology Letters* **266**: 10-19.
- 6 Lindeberg G, Lindeberg M, Lundgren L, Popoff T, Theander O, 1980. Stimulation
7 of litter-decomposing basidiomycetes by flavonoids. *Transactions of the*
8 *British Mycological Society* **75**: 455-459.
- 9 Nilsson T, Daniel G, Kirk TK, Obst JR, 1989. Chemistry and microscopy of wood
10 decay by some higher ascomycetes. *Holzforschung* **43**: 11-18.
- 11 Okane I, Nakagiri A, 2007. Taxonomy of an anamorphic xylariaceous fungus from
12 a termite nest found together with *Xylaria angulosa*. *Mycoscience* **48**:
13 240-249.
- 14 Okane I, Srikitikulchai P, Toyama K, Læssøe T, Sivichai S, Hywel-Jones N,
15 Nakagiri A, Potacharoen W, Suzuki K, 2008. Study of endophytic
16 Xylariaceae in Thailand: diversity and taxonomy inferred from rDNA
17 sequence analyses with saprobes forming fruit bodies in the field.

- 1 Mycoscience **49**: 359-372.
- 2 Osono T, 2007. Ecology of ligninolytic fungi associated with leaf litter
3 decomposition. Ecological Research **22**: 955-974.
- 4 Osono T, Takeda H, 2002. Comparison of litter decomposing ability among diverse
5 fungi in a cool temperate deciduous forest in Japan. Mycologia **94**: 421-427.
- 6 Osono T, Fukasawa Y, Takeda H, 2003. Roles of diverse fungi in larch needle litter
7 decomposition. Mycologia **95**: 820-826.
- 8 Osono T, Hobara S, Koba K, Kameda K, 2006. Reduction of fungal growth and
9 lignin decomposition in needle litter by avian excreta. Soil Biology &
10 Biochemistry **38**: 1623-1630.
- 11 Osono T, Ishii Y, Hirose D, 2008. Fungal colonization and decomposition of
12 *Castanopsis sieboldii* leaves in a subtropical forest. Ecological Research **23**:
13 909-917.
- 14 Osono T, Ishii Y, Takeda H, Seramethakun T, Khamyong S, To-Anun C, Hirose D,
15 Tokumasu S, Kakishima M, 2009. Decomposition and fungal succession on
16 *Shorea obtusa* leaf litter in a tropical seasonal forest in northern Thailand.
17 Fungal Diversity **36**: 101-119.

- 1 Pointing SB, Parungao MM, Hyde KD, 2003. Production of wood-decay enzymes,
2 mass loss and lignin solubilization in woody by tropical Xylariaceae.
3 Mycological Research **107**: 231-235.
- 4 Pointing SB, Pelling AL, Smith GJD, Hyde KD, Reddy CA, 2005. Screening of
5 basidiomycetes and xylariaceous fungi for lignin peroxidase and laccase
6 gene-specific sequences. Mycological Research **109**: 115-124.
- 7 Rodrigues KF (1994) The foliar fungal endophytes of the Amazonian palm
8 *Euterpe oleracea*. Mycologia **86**: 376-385.
- 9 Rodrigues KF, Leuchtman A, Petrini O, 1993. Endophytic species of *Xylaria*:
10 cultural and isozymic studies. Sydowia **45**: 116-138.
- 11 Rogers JD, 2000. Thoughts and musings on tropical Xylariaceae. Mycological
12 Research **104**: 1412-1420.
- 13 Rogers JD, Callan BE, Samuels GJ, 1987. The Xylariaceae of the rain forests of
14 north Sulawesi (Indonesia). Mycotaxon **29**: 133-172.
- 15 Rogers JD, Callan BE, Rossman AY, Samuels GJ, 1988. *Xylaria* (Sphaeriales,
16 Xylariaceae) from Cerro de la Neblina, Venezuela. Mycotaxon 31: 103-153.
- 17 Rogers JD, Ju YM, Lehmann J, 2005. Some *Xylaria* species on termite nests.

- 1 Mycologia **97**: 914-923.
- 2 Sinsabaugh RL, Carreiro MM, Repert DA, 2002. Allocation of extracellular
3 enzymatic activity in relation to litter composition, N deposition, and mass
4 loss. Biogeochemistry **60**: 1-24.
- 5 Van der Gucht K, 1996. *Xylaria* species from Papua New Guinea: cultural and
6 anamorphic studies. Mycotaxon **60**: 327-360.
- 7 Visser AA, Ros VID, de Beer ZW, Debets AJM, Hartog E, Kuyper TW, Læssøe T,
8 Slippers B, Aanen DK, 2009. Levels of specificity of *Xylaria* species
9 associated with fungus-growing termites: a phylogenetic approach.
10 Molecular Ecology **18**: 553-567.
- 11 Waldrop MP, Zak DR, 2006. Response of oxidative enzyme activities to nitrogen
12 deposition affects soil concentrations of dissolved organic carbon.
13 Ecosystems **9**: 921-933.
- 14 Worrall JJ, Anagnost SE, Zabel RA, 1997. Comparison of wood decay among
15 diverse lignicolous fungi. Mycologia **89**: 199-219.
- 16 Whalley AJS, 1993. Tropical Xylariaceae: their distribution and ecological
17 characteristics. In: Issac S, Frankland JC, Watling R, Whalley AJS (Eds.),

- 1 Aspects of Tropical Mycology. Cambridge University Press, Cambridge, pp.
2 103-119.
- 3 Whalley AJS, 1996. The xylariaceous way of life. *Mycological Research* **100**:
4 897-922.
- 5 Whalley AJS, 1997. Xylariaceae. In: Kevin H (Ed.), *Biodiversity of Tropical*
6 *Microfungi*. Hong Kong University Press, Hong Kong, pp. 279-296
- 7 White TJ, Bruns T, Lee S, Taylor JW, 1990. Amplification and direct sequencing of
8 fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH,
9 Sninsky JJ, White TJ (Eds.), *PCR Protocols: a Guide to Methods and*
10 *Applications*. Academic Press, New York, USA, pp. 315-322
- 11

1 Figure legend

2

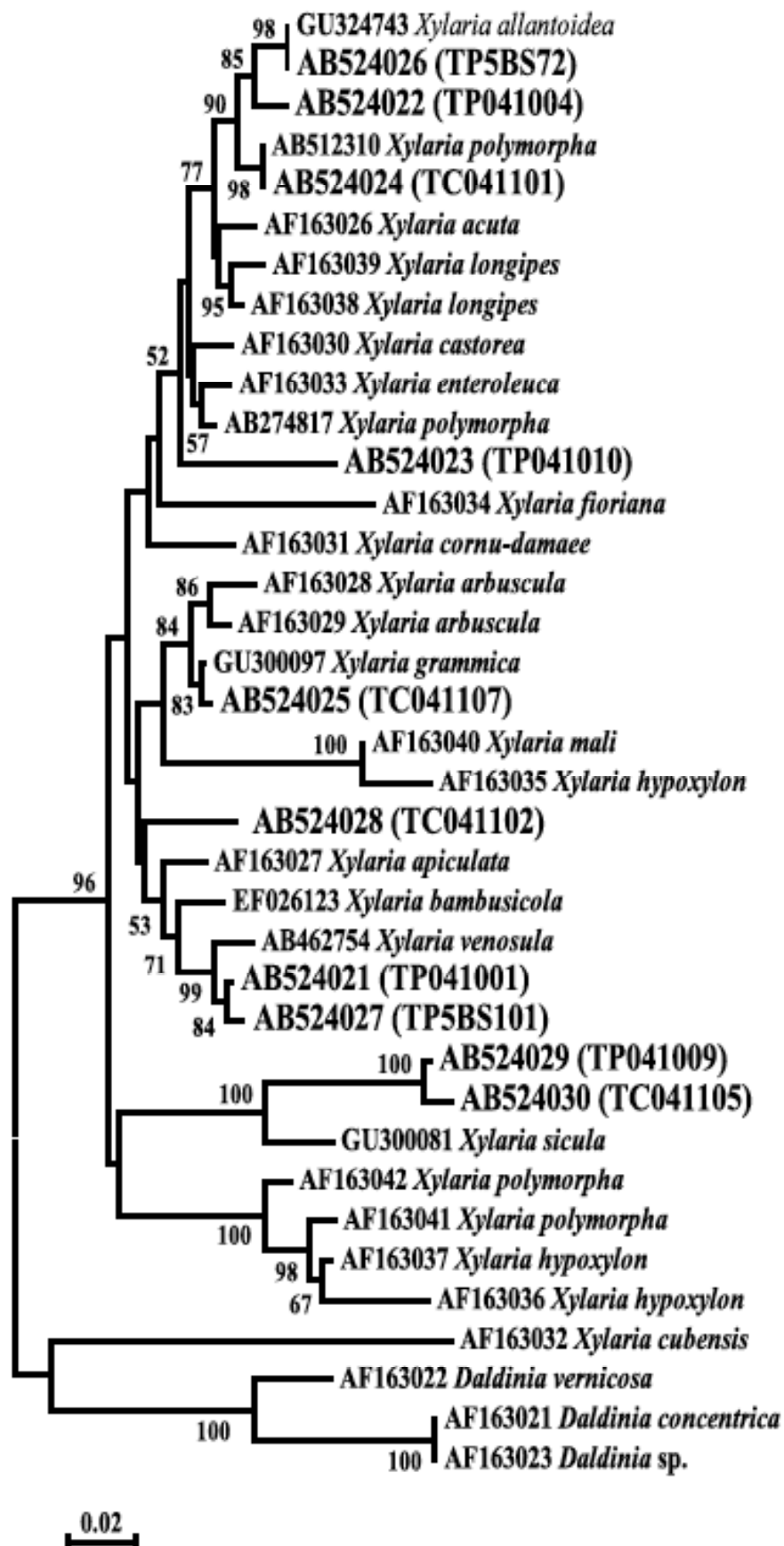
3 Fig 1 - Neighbour-joining tree based on the rDNA ITS sequences of the *Xylaria*
4 species used in the present study. Twenty sequences used in Lee et al. (2000) and
5 best identified BLAST match taxa were also included in the phylogenetic analysis.
6 Bootstrap values above 50% are given adjacent to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

7

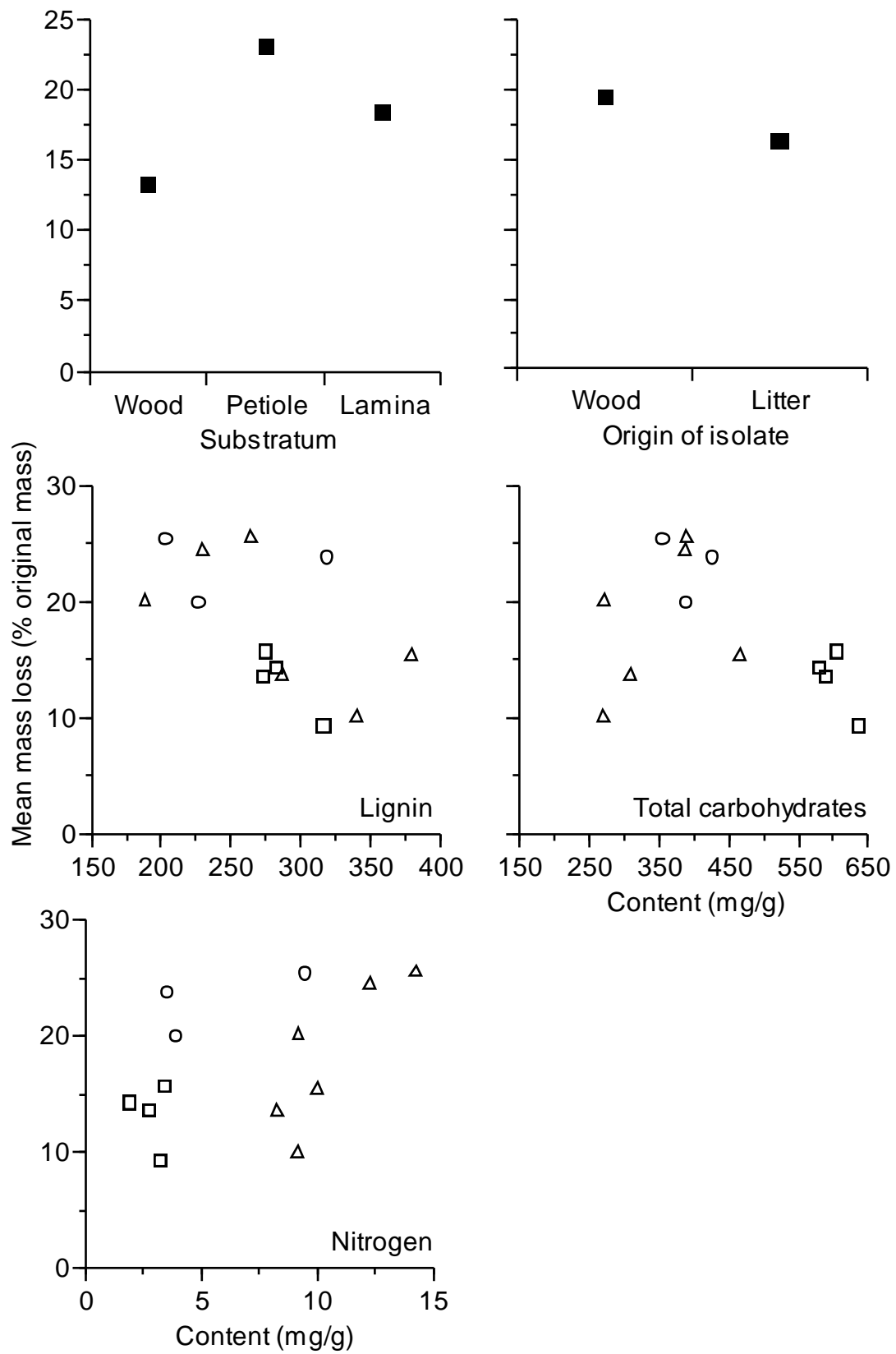
8 Fig 2 - Mean values of mass loss for wood, petiole, and lamina (upper, left) and
9 wood and litter isolates (upper, right) and relationship between the mean value of
10 mass loss for 10 *Xylaria* isolates and the initial contents of lignin (middle, left),
11 total carbohydrate (middle right), and nitrogen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

13

14



1 Osono et al. Fig 2



1 Osono et al. Table 1

2

3

4 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	<i>Xylaria venosula</i> (AB462754)	96
TP041004	NU	Oct	Branch	MA	AB524022	<i>Xylaria allantoidea</i> (GU324743)	97
TP041010	NU	Oct	Branch	MA	AB524023	<i>Xylaria polymorpha</i> (AB274817)	93
TC041101	QSBG	Nov	Log	MA	AB524024	<i>Xylaria polymorpha</i> (AB512310)	99
TC041107	DS	Nov	Log	MA	AB524025	<i>Xylaria grammica</i> (GU300097)	98
Litter isolates							
TP5BS72	NU	Oct	Lamina	SD	AB524026	<i>Xylaria allantoidea</i> (GU324743)	99
TP5BS101	NU	Oct	Lamina	SD	AB524027	<i>Xylaria venosula</i> (AB462754)	96
TC041102	QSBG	Nov	Petiole	MA	AB524028	<i>Xylaria bambusicola</i> (EF026123)	93
TP041009	NU	Oct	Petiole	MA	AB524029	<i>Xylaria sicula</i> (GU300081)	91
TC041105	DS	Nov	Vein	MA	AB524030	<i>Xylaria sicula</i> (GU300081)	91

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

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

1 Osono et al. Table 2

2

3

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

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

7

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										
<i>Shorea</i>	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
<i>Quercus</i>	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
<i>Dipterocarpus</i>	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
<i>Tectona</i>	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										
<i>Bauhinia</i>	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
<i>Macalanga</i>	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
<i>Dipterocarpus</i>	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										
<i>Bauhinia</i>	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
<i>Tectona</i>	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
<i>Macalanga</i>	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
<i>Pinus</i>	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
<i>Shorea</i>	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
<i>Dipterocarpus</i>	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

1

2

1 Osono et al. Table 4

2

3

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

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

<i>Castanopsis sieboldii</i>	nd	nd	13.5 (1)	13.8 (1)	W	EG	20	12	9
<i>Shorea obtusa</i>	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	

1 * *Xylaria carpophila* inhabiting cupules.

2 nd not determined.

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

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

5 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
6 (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
7 study.