

Fungal biomass in decayed wood

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Abstract: Fungal biomass in decayed wood was measured using glucosamine as an indicator. Apparent mass loss and glucosamine content of wood blocks decayed by *Trametes versicolor*, *Oligoporus placentus*, *Bjerkandera adusta*, *Gloeophyllum trabeum*, and *Phialocephala dimorphospora* were measured at selected intervals. After 12 weeks of decay by *T. versicolor*, birch blocks contained 31.3% fungal biomass, more than any other fungus-wood combination. Fungal biomass in birch decayed by *B. adusta* and *G. trabeum* exceeded 9.0%. Measurements of apparent mass loss underestimate actual loss of wood mass, determined by correcting for fungal biomass, by as much as 42%. Such errors are greatest at lower mass losses.

Key Words: chitin, fungal biomass, glucosamine, wood decay

INTRODUCTION

Mass loss of wood is the most commonly used measure of wood decay in laboratory studies. Such studies neglect the mass of the fungus present in decayed wood. We are aware of only a few cases in which fungal biomass was acknowledged in studies of wood decay. Cowling (1961), in a study of the chemical and physical changes in wood during decay, acknowledged that fungal biomass is a source of error in mass loss measurements but considered it to be negligible. Swift (1973) quantified fungal biomass in wood decayed by *Trametes versicolor* (L.: Fr.) Pilat. Sawdust (*Castanea sativa* Mill.) decayed by *T. versicolor* for 12 wk was found to contain 47% fungus. We have calculated the actual mass loss based on Swift's results and determined that it indicates a 45% error in measurement of apparent mass loss. Using the results of Gurusiddiah et al. (1978) we calculated 5.3% fungus in coniferous wood chips decayed by *T. versicolor* for 5 months. Thus, considerable

errors in apparent mass loss may occur because of fungal biomass in decayed wood but the size of these errors has not been investigated; nor has the variation among fungi, decay types, and wood types been explored. Our study was designed to quantify fungal biomass in decayed wood and to estimate the contributions of residual wood vs. fungal biomass to total dry mass in decayed wood samples at various stages and conditions of decay.

To establish a reliable measure of fungal biomass in decayed wood, one can measure a chemical component that is present in fungi but not in wood and calculate a factor that accounts for the concentration of the component in pure mycelia. Chitin is one of the most commonly used components for estimating fungal biomass (Matcham et al., 1985; Mayama et al., 1975; Ride and Drysdale, 1971, 1972; Swift, 1973). Chitin, an unbranched polymer of N-acetyl-D-glucosamine, is a cell wall component of some fungi. Acid hydrolysis of chitin releases glucosamine and the ratio of glucosamine to dry mass in pure mycelium can be determined and used to calculate fungal biomass in the substrate. This approach has been applied to a wide range of substrates including plant tissue (Matcham et al., 1985; Mayama et al., 1975; Ride and Drysdale, 1971, 1972; Toppan et al., 1976; Wu and Stahman, 1975), decayed wood (Gurusiddiah et al., 1978; Swift, 1973), mycorrhizae (Becker and Gerdemann, 1977; Bethlenfalvai et al., 1981; Hepper, 1977; Johnson and McGill, 1990; Toth et al., 1991), fermentations (Desgranges et al., 1991), and food products (Arima and Uozumi, 1967; Sakurai et al., 1977).

MATERIALS AND METHODS

Cultures.—The following fungal cultures were selected as representatives of white-, brown-, and soft-rot fungi: *Trametes versicolor* (491-FS), *Oligoporus placentus* (Fr.) Gilbn. & Ryvarden (622), *Bjerkandera adusta* (Willd.: Fr.) P. Karst. (620), *Gloeophyllum trabeum* (Fr.) Murr. (621), and *Phialocephala dimorphospora* Kendrick (N-18; kindly provided by C. J. K. Wang). Fungi were cultured on malt extract agar (1.5%) and incubated at 25 C.

Decay tests.—Yellow birch (*Betula alleghaniensis* Britton) and southern yellow pine (*Pinus taeda* L.) were subjected to decay by various fungi using a modified

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soil-block procedure (Anonymous, 1981). Sufficient deionized water was added to each glass bottle to obtain a soil moisture content of 44.2 ml water per 100 g dry soil (equivalent to 130% of moisture-holding capacity). Approximately 240 cm³ (equivalent to 272 g dry soil) soil (sandy loam) were added to each bottle (500 ml capacity). Oven-dried (103 C, 24 h) and weighed test blocks (20 × 10 × 5 mm) and wood feeder strips (3 × 25 × 33 mm) were soaked in deionized water for 5 min. Two feeder strips were then placed side-by-side on top of the soil in each bottle. Six test blocks were placed on top of the feeder strips. Each bottle was capped, autoclaved for 75 min, cooled, and inoculated with six agar plugs of mycelium. Plugs (5 mm diam) were taken from actively growing margins of approximately 1-wk-old malt extract cultures. Seven bottles were used per treatment.

At the time of harvest (3, 6, 9, or 12 wk), surface mycelium was removed from blocks with a razor blade and forceps. Mycelium collected from the surface of the test blocks in each chamber was lyophilized and stored at 4 C until analysis by high pressure liquid chromatography (HPLC). Test blocks were oven dried and weighed. Apparent mass loss (*A*) was calculated using the following formula: $A (\%) = [(OW - DW) \times 100] / OW$, where *OW* = original dry mass and *DW* = post-decay dry mass.

Sample preparation, hydrolysis, and glucosamine content.—All decayed wood and mycelial samples were ground in a Wiley mill to pass a 40-mesh screen (opening size 0.42 mm). Moisture content of a subsample was determined and samples of 200 ± 10 mg (equivalent dry mass) were dried overnight in a vacuum oven (38 C) in tared hydrolysis tubes. The dry mass of each sample was then measured.

Hydrolysis followed the procedure for determination of Klason (acid-insoluble) lignin (Kirk and Obst, 1988). This procedure was chosen because it would allow for simultaneous determination of glucosamine, lignin, and wood sugars. Each sample was hydrolyzed with 2 ml 72% (w/w) H₂SO₄ and stirred frequently in a water bath at 30 C. At the end of 1 h, each sample was diluted with 56 ml of water. Tubes were then covered with foil and autoclaved at 120 C for 1 h. One milliliter of glucosamine hydrochloride (Sigma) standard (1 mg/ml) was dried in the vacuum oven and subjected to the identical hydrolysis procedure with each batch of samples.

Immediately after autoclaving, each sample was poured and rinsed through a glass microfiber filter (Whatman 934-AH) in crucibles. One ml of fucose internal standard (20 mg/ml) was added to the filtrate, which was then diluted with water to a total volume of 100 ml. An aliquot was filtered through an On-

Guard-H cartridge (Dionex), followed by a 0.2- μ m nylon Acrodisc filter (Gelman Sciences). Three volumes of this filtered aliquot were diluted with 7 volumes of water to bring concentrations to a range appropriate for chromatography by HPLC.

Analytical conditions.—The HPLC equipment and conditions (Dionex) were similar to that of Pettersen and Schwandt (1991) and consisted of a gradient pump, pulsed amperometric detector, post-column reagent module, eluant degas module, 4400 integrator, and a CarboPac PA1 column and guard column. Working electrode potentials (pulse duration) were: $E_1 = 0.05$ V (480 ms), $E_2 = 0.6$ V (120 ms), $E_3 = -0.6$ V (60 ms). Two hundred microliters of sample were flushed through a 25- μ l injection loop before sample injection into the eluant stream. The glucosamine standard was injected initially and after every six samples.

Glucosamine was quantified using 15 mM NaOH as an eluant at a flow rate of 1 ml/min. The output range of the detector was 3000 nA after injection and was lowered to 100 nA for increased sensitivity after elution of the internal standard. The total time of the separation was 35 min, including 15 min of sample run after injection, 10 min of column regeneration, and 10 min of baseline stabilization with eluant. A postcolumn addition of 300 mM NaOH (0.7 ml/min) was used to increase baseline stability. Each sample was injected three times and the mean was used in all calculations.

The ratio of glucosamine to dry mass in pure mycelium growing on the wood blocks in each chamber was determined and used to calculate fungal biomass in associated blocks. Glucosamine in sound yellow birch and sound southern yellow pine was quantified and subtracted from that in decayed samples to correct for minute amounts of glucosamine in sound wood.

RESULTS

Fungal analysis.—Glucosamine content of pure mycelia from wood surfaces ranged from 1.29% (w/w; *P. dimorphospora* removed from birch) to 3.11% (*G. trabeum* removed from birch) (TABLE I). Glucosamine content (mean ± standard error) of *T. versicolor* mycelia at 3, 6, 9, and 12 wk was 1.82 ± 0.02%, 1.58 ± 0.06%, 1.70 ± 0.07%, and 2.28 ± 0.06%, respectively. *Trametes versicolor*, when grown on malt extract broth and agar for 3 wk contained 0.33% and 0.53% glucosamine, respectively. Glucosamine content of *T. versicolor* grown for 2 wk on potato dextrose agar was 1.31%.

Fungal biomass and mass loss.—Small amounts of glucosamine were detected in sound wood samples. Sound yellow birch had 0.024 ± 0.003% glucosamine and

southern yellow pine had $0.017 \pm 0.002\%$ glucosamine. Even a collection of sound *Pinus palustris* Mill., frozen immediately after felling of a live tree, had $0.009 \pm 0.001\%$ glucosamine. These values for glucosamine in sound wood were subtracted from those in decayed samples.

After 12 wk, biomass of three of the five fungi grown on birch (*T. versicolor*, *B. adusta* and *G. trabeum*) exceeded 9.0% of the residual block mass (FIG. 1). Birch blocks decayed by *T. versicolor* for 12 wk contained 31.3% fungal biomass, the most of all decayed wood samples analyzed (FIGS. 1, 2).

Biomass of *T. versicolor* in birch and pine increased with time, and that in birch was higher than that in pine for all dates (FIG. 2). Similarly, apparent and actual mass losses of birch and pine decayed by *T. versicolor* increased with time, and values for birch were higher than those for pine at all dates (FIG. 3). Percent error in apparent mass loss measurements (based on actual mass loss measurements) of pine and birch decayed by *T. versicolor* decreased during decay (FIG. 2).

In general, biomass of *O. placentus* in wood also increased with time (FIG. 2). *Oligoporus placentus* biomass in pine exceeded that in birch for all dates except 3 wk (FIG. 2). Similarly, apparent and actual mass losses of pine decayed by *O. placentus* were higher than that of birch samples for all dates except 3 wk (FIG. 3). As with *T. versicolor*, percent error in apparent mass loss measurements of birch decreased as decay increased (FIG. 2). Percent error in apparent mass loss measurements of pine increased initially (up to 6 wk of decay by *O. placentus*), but decreased for the remainder of the decay study (FIG. 2).

DISCUSSION

Fungal analysis.—An assumption of this study is that glucosamine content of mycelium in wood is the same as that in the pure mycelium that was analyzed. The use of chitin for estimation of fungal biomass has been questioned by several researchers because of conflicting reports regarding the variation of mycelial chitin content with age (Arima and Uozumi, 1967). Also, chitin content of mycelia in the natural substrate may be different from that in culture (Bartnicki-Garcia, 1968; Johnson and McGill, 1990; Martin et al., 1990; Ride and Drysdale, 1972; Seitz et al., 1979; Sharma et al., 1977). In this study, glucosamine concentrations in mycelia remained relatively constant during fungal development. These results agree with those of Desgranges et al. (1991) and of Ride and Drysdale (1972) regarding shaken fungal cultures. The experimental design of the study provided realistic conditions for comparative measurements because the aerial myce-

TABLE I. Percent glucosamine in various fungi grown on different substrates

Fungus	No. of replicates	Substrate	Age of mycelium (wk)	Glucosamine in mycelium (%) ^a
<i>T. versicolor</i>	26	Birch	3, 6, 9, 12	1.87 ± 0.06
	3	Pine	9, 12	1.97 ± 0.23
<i>O. placentus</i>	4	Birch	9, 12	2.72 ± 0.06
	2	Pine	9, 12	2.16 ± 0.61
<i>G. trabeum</i>	2	Birch	12	3.11 ± 0.09
<i>B. adusta</i>	9	Birch	12	1.71 ± 0.03
<i>P. dimorphospora</i>	1	Birch	12	1.29 ^b

^a Mean \pm standard error.

^b Based on one replicate.

lium grown on the wood to be analyzed was harvested for each analysis. Thus, the nutritional source for the control mycelium was nearly identical to that in wood. Also, mycelia grown on other media exhibited lower glucosamine contents (TABLE I), so using the figures from wood-grown mycelia gives the most conservative estimate of fungal biomass in wood.

The glucosamine content of *T. versicolor* mycelia removed from birch blocks (1.87%) was comparable to, but higher than, the glucosamine content measured in *T. versicolor* mycelia removed from chestnut sawdust (1.2%) (Swift, 1973). The concentration of glucosamine in mycelia from *T. versicolor* removed from pine (1.97%, TABLE I) was also comparable to, but higher than, that calculated from earlier work by Gurusiddaiah et al. (1978) (1.0% glucosamine in mycelia of *T. versicolor* removed from a coniferous wood substrate). In this study, the brown rot fungus, *O. placentus*, contained 2.16% glucosamine when removed from pine. As with *T. versicolor*, this value is higher than that calculated from the results of Gurusiddaiah et al. (1978) which indicated 0.56% glucosamine in mycelia from *O. placentus* grown on a coniferous wood substrate. Differences among the glucosamine values obtained in these studies could be attributed to the culture media used. Gurusiddaiah et al. (1978) used a wood chip mixture of western white pine, fir, spruce, and western red cedar, and Swift (1973) used chestnut sawdust as opposed to the southern yellow pine and yellow birch used in this study. Also, the analytical methods, which varied greatly among the studies, may differ in sensitivity and glucosamine destruction during hydrolysis.

Fungal biomass and mass loss.—The small amounts of glucosamine detected in sound wood samples may be a result of a minor wood component that contains glucosamine or a small amount of fungus in the wood before it was harvested. Detection of glucosamine in

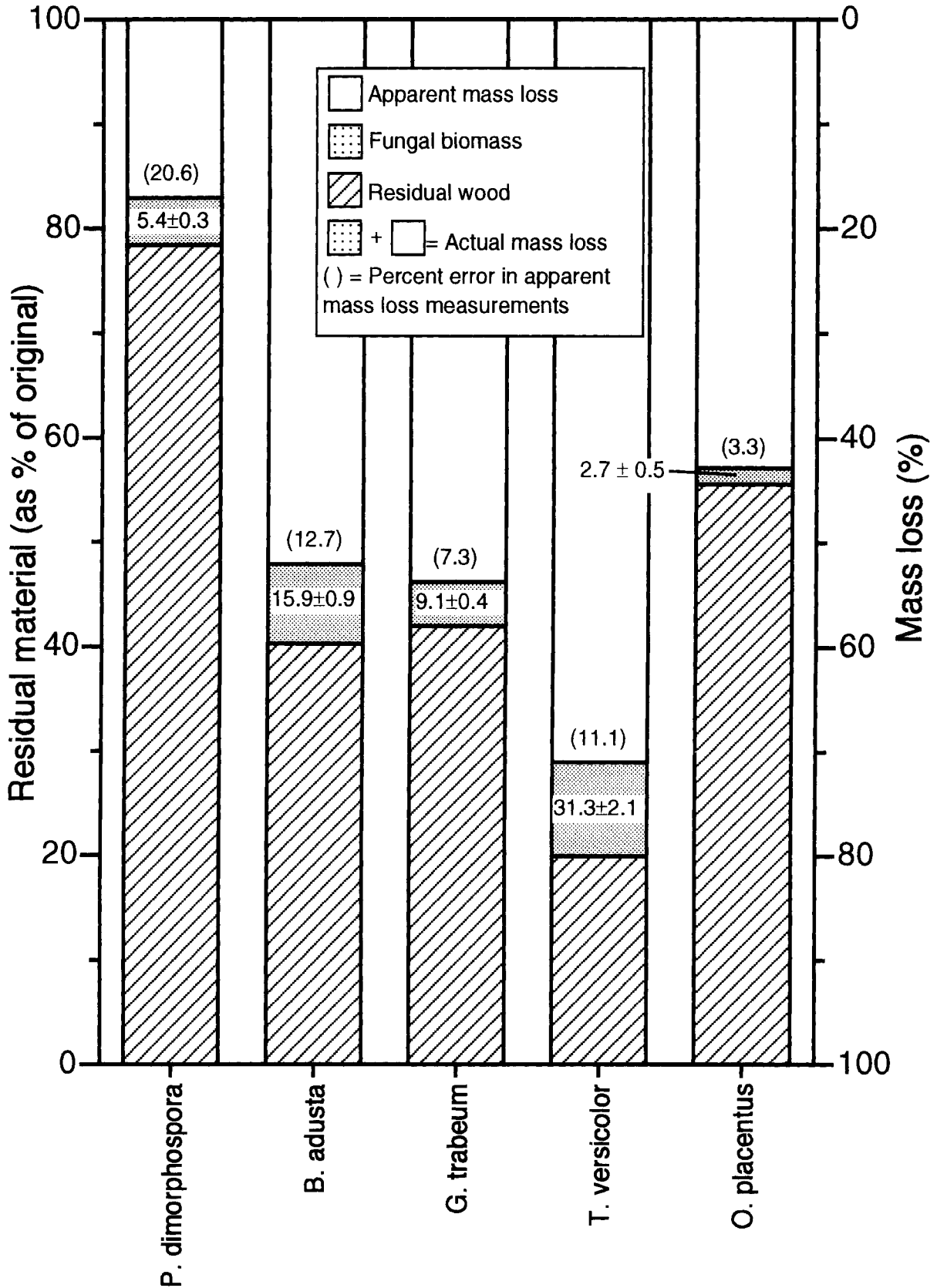


FIG. 1. Relationship between apparent and actual mass loss and fungal biomass for birch decayed by five fungi for 12 wk. The stippled areas indicate fungal biomass as a percent of original wood mass; the value inside represents fungal biomass as a percent of residual material mass.

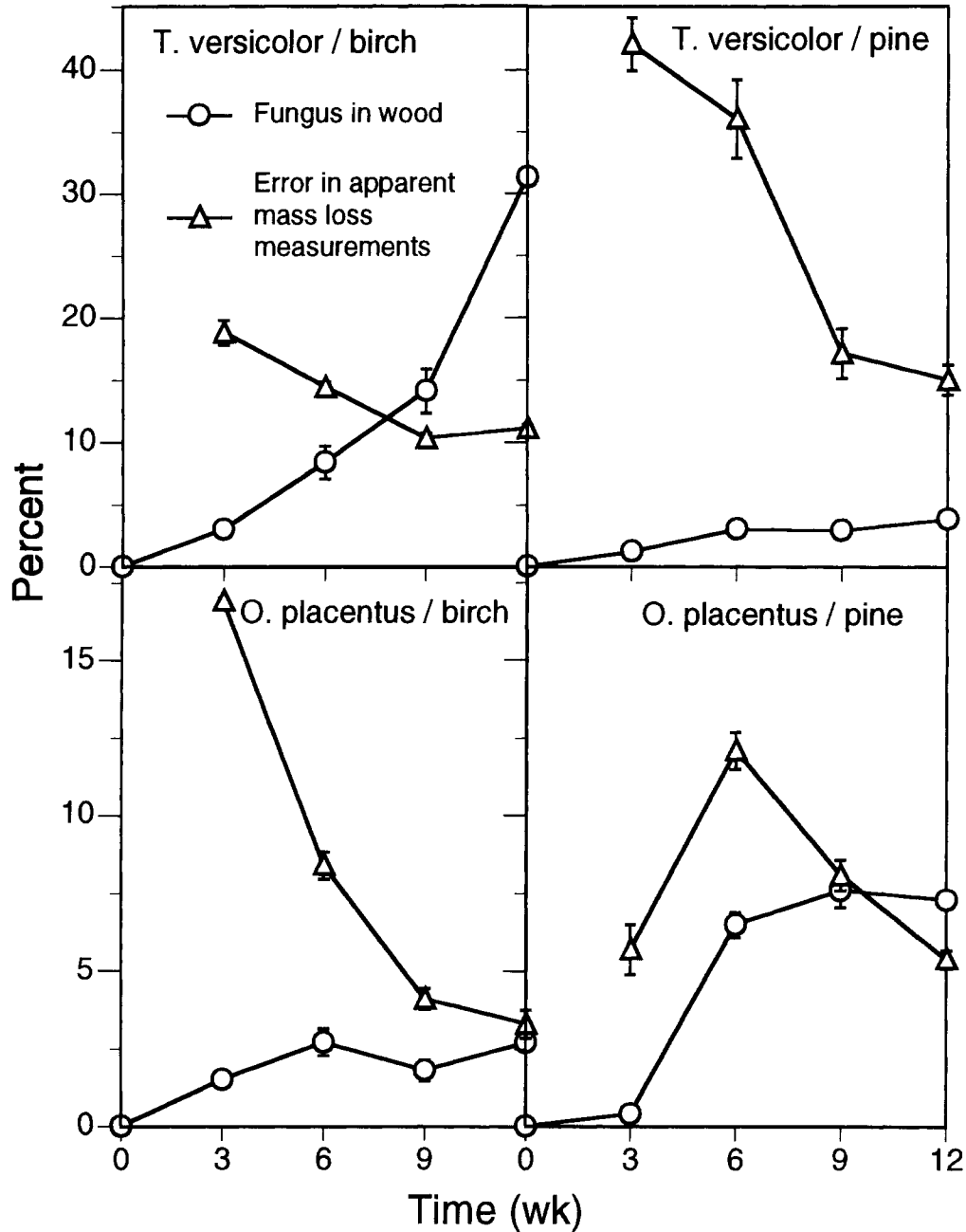


FIG. 2. Fungal biomass and estimated error in measurements of apparent mass loss of birch and pine decayed by *Trametes versicolor* and *Oligoporus placentus*. Fungal biomass is expressed as percent of the mass of the residual material. Note that the scaling of the y-axis differs between the two tiers. Error bars represent standard error.

sound wood could also be attributed to the presence of bacteria or actinomycetes that have peptidoglycan cell walls, of which N-acetylglucosamine is a constituent. Other researchers have also detected small amounts of glucosamine in sound wood samples (Gurusiddaiah et al., 1978) and other plant tissues (Mayama et al., 1975; Ride and Drysdale, 1971; Rotter, 1989).

It is possible that glucosamine detection efficiency is different in pure mycelium vs. decayed wood. However, it was found that detection of sugars was not

significantly affected by presence of wood (Worrall and Anderson, 1993).

Our values for fungal biomass in birch samples decayed by *T. versicolor* are lower than those calculated from the results of Swift (1973). After 3, 6, 9, and 12 wk of decay by *T. versicolor*, calculations based on Swift's results indicate 23.6, 29.1, 35.7, and 47.1% fungus in decayed chestnut sawdust, respectively. Differences in fungal biomass values obtained in this study and those obtained by Swift (1973) may be attributed to the dif-

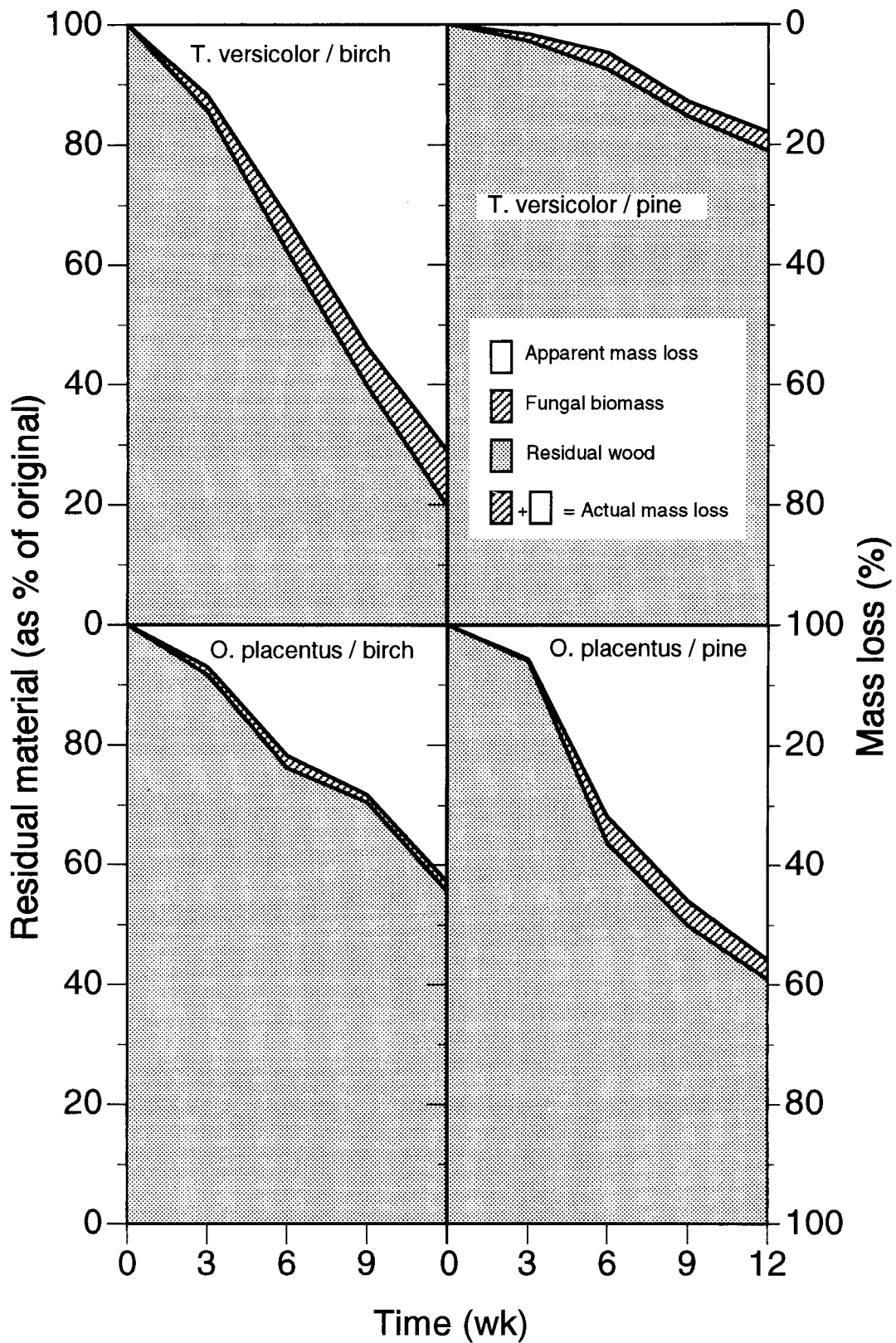


FIG. 3. Relationship between apparent and actual mass loss and fungal biomass for birch and pine decayed by *Trametes versicolor* and *Oligoporus placentus* for 3, 6, 9, and 12 wk.

ferent species (birch vs. chestnut) and size (blocks vs. sawdust) of wood samples used in the decay studies. More mycelium may be expected to accumulate in spaces between sawdust particles than in a solid block of the same wood.

In this study, pine blocks decayed by *O. placentus* for 12 wk were found to contain 7.3% fungus. Pine decayed by *T. versicolor* for 12 wk was found to contain 3.8% fungus (FIG. 2). These results are in the range of those calculated from results of Gurusiddaiah et al. (1978) who found 11.8% and 5.4% fungus in coniferous chips decayed by *O. placentus* and *T. versicolor*, respectively, for 5 months. These differences may be attributed to the differences in length of decay and decay substrates.

Fungal biomass contributed a substantial portion of the mass of decayed samples, introducing considerable errors in measurements of apparent mass loss of wood in many fungus-wood combinations. Percent error in apparent mass loss measurements was highest at lower mass losses although errors were often substantial even at higher mass losses. Errors in apparent mass loss measurements of pine decayed by *T. versicolor* for 3 wk and 6 wk were 42.0 and 36.0%, respectively. Errors in apparent mass loss measurements of birch decayed by *T. versicolor* and *O. placentus* for 3 wk exceeded 16% (FIG. 2). In all cases, actual mass loss was higher than the apparent mass loss of wood. Therefore, the apparent mass loss is a conservative estimate of wood decay, which should be taken into account when interpreting results of wood decay studies.

Fungal biomass contributes to the mass and therefore to the chemical components of decayed samples. This implies that a) in chemical analyses of wood, components in the mycelium (such as fungal carbohydrates) are analyzed as wood components; b) for biomass conversion projects, mass of decayed wood overestimates the amount of useful wood components; c) for wood decay studies related to wood products utilization, preservatives, utility pole lifespan, etc., mass loss during decay underestimates the degradation of wood components.

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