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Comparison of Degradative Ability, Enzymatic Activity, and Palatability of Aquatic Hyphomycetes Grown on Leaf Litter

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Stream fungi have the capacity to degrade leaf litter and, through their activities, to transform it into a more palatable food source for invertebrate detritivores. The objectives of the present study were to characterize various aspects of fungal modification of the leaf substrate and to examine the effects these changes have on leaf palatability to detritivores. Fungal species were grown on aspen leaves for two incubation times. Leaves were analyzed to determine the weight loss, the degree of softening of the leaf matrix, and the concentrations of ATP and nitrogen associated with leaves. The activities of a protease and 10 polysaccharide-degrading enzymes produced by each fungus were also determined. Most fungi caused similar changes in physicochemical characteristics of the leaves. All fungi exhibited the capability to depolymerize pectin, xylan, and cellulose. Differences among fungi were found in their capabilities to produce protease and certain glycosidases. Leaf palatability was assessed by offering leaves of all treatments to larvae of two caddisfly shredders (*Trichoptera*). Feeding preferences exhibited by the shredders were similar and indicated that they perceived distinct differences among fungi. Two fungal species were highly consumed, some moderately and others only slightly. No relationships were found between any of the fungal characteristics measured and detritivore feeding preferences. Apparently, interspecific differences among fungi other than parameters associated with biomass or degradation of structural polysaccharides influence fungal palatability to caddisfly detritivores.

During the initial stages of leaf litter decomposition in streams, microbial colonization alters the chemical and physical nature of the leaf resource. Leaves become softened, their nitrogen and ATP contents rise, and respiratory rates and activities of degradative enzymes associated with the leaf matrix increase (17, 22, 24, 27, 31; F. J. Triska, Ph.D. thesis, University of Pittsburgh, Pittsburgh, Pa., 1970). The palatability of most types of leaf detritus to stream detritivores also improves during this period of microbial colonization (1, 13). This observation, together with studies demonstrating that detritivores preferentially feed on microbially colonized versus sterile leaves (4, 5, 17, 18, 20), suggests that there are interactions between detritivore feeding and microbial colonization. Further, for the shredder functional group of stream invertebrates (coarse particle feeders [12]), fungi increase the palatability of detritus significantly more than bacteria (17, 18, 20). Characteristics of fungi themselves (high nutritional value, e.g., higher concentration of nitrogen) and fungal modification of the leaf resource (enzymatic hydrolysis to a more digestible form) have been implicated as bases for these trophic interactions (7, 8, 13).

Various fungal species can be isolated from leaf detritus and shown to increase its palatability over uncolonized leaves (4, 17). However, leaves colonized by different fungal species are not equally palatable to detritivores. Barlocher and Kendrick (4) found that an amphipod detritivore feeds preferentially on leaves colonized by certain fungal species when offered choices among five terrestrial and five aquatic hyphomycetes. However, comparative studies of the fungal species associated with leaves in streams show that the fungi active in this habitat are aquatic hyphomycetes (6, 25). Thus, stream invertebrates feeding on leaf detritus typically encounter aquatic hyphomycetes more frequently than other types of fungi.

If selective feeding on leaves colonized by different fungal species is widespread among detritivores, then these interactions represent potentially important factors regulating both fungal populations and leaf litter processing in streams. Before the role of selective detritivory in the regulation of fungal community structure and function can be assessed, it is necessary to determine the feeding preferences of detritivores. One objective of the present study was to document patterns of selective feeding by cad-

disfly larvae (*Trichoptera*), the major shredders in a mountain stream, for the aquatic hyphomycete species commonly found on leaves in that stream. In the examination of these fungus-invertebrate interactions, it would also be desirable to identify specific differences among fungi which account for their differential palatability to shredders. With respect to such interactions, it would appear that the array of extracellular degradative enzymes produced by these fungi may be of central importance (8, 13). The inability of certain species to produce one or more types of enzymes would prevent the degradation of specific leaf polymers. This would affect not only the growth but also the physical and chemical modification of leaf tissue by a fungus in comparison with other species possessing these enzymes. Because information on the degradative capabilities of some of these fungi is lacking, a second objective of this study was to compare these aquatic hyphomycete species with respect to growth, degradation, and types of extracellular enzymes produced during colonization of leaf litter.

Current ideas concerning fungus-detritivore interactions (7, 8, 13) suggest that selective feeding by detritivores should be related to differences in parameters associated with biomass accumulation or degradative capabilities of the fungi. Therefore, our experimental hypothesis couples our two objectives and proposes that differences in palatability of leaves colonized by fungal species would be due to differential degradation, growth, or extracellular enzyme production by these fungi. Experiments were designed to permit leaves colonized by each species to be analyzed for changes in softness, weight loss, and ATP and nitrogen content. Culture filtrates were assayed for types of enzyme activity. Replicate leaves from each culture were simultaneously offered to caddisfly detritivores to assess feeding preferences. Consumption of leaves colonized by each of the 10 fungal species were compared to identify which fungus-mediated changes, if any, represented potential mechanisms allowing detritivores to discriminate among leaves colonized by different fungi as food sources.

MATERIALS AND METHODS

Fungal isolates. Nine of the aquatic hyphomycete species were obtained as single spore isolates from leaf litter collected from a stream in the Sacramento Mountains of New Mexico. These included *Alatospora acuminata* Ingold, *Anguillospora pseudolongissima* Ranzoni, *Articulospora inflata* Ingold, *Clavariopsis aquatica* de Wild, *Flagellospora curvula* Ingold, *Heliscus lugdunensis* Sacc. and Therry, *Lemonniera aquatica* de Wild., *L. terrestris* Tubaki, and *Tetracladium marchalianum* de Wild. *Tetrachaetum elegans* Ingold was isolated from leaves collected from a stream in the Cascade Range in Oregon.

Leaf cultures. Aspen (*Populus tremuloides* Michx.) leaves were gathered shortly after leaf abcission in October, 1980, air dried, and stored until needed. Before each experiment, the petioles were removed, and leaves were leached at 5°C for 5 days with daily changes of distilled water. Leaves were individually tagged with numbered, Monel, size 1, mouse ear tags (Kentucky Band and Tag Co.), oven dried at 45°C, and weighed to the nearest 0.1 mg. Leaves for each culture were placed in separate Whirlpak bags. Bags of leaves were sterilized with gamma irradiation (2 to 3 megarads).

Sterile leaves were added to Fernbach flasks (2,800 ml) containing 1.5 liters of a sterile, inorganic salt solution (KNO_3, 1.01 g; NaCl, 0.11 g; KH_2PO_4, 0.41 g; K_2HPO_4, 0.52 g; $MgSO_4 \cdot 7H_2O$, 0.49 g; $CaCl_2 \cdot 2H_2O$, 0.15 g; distilled water, 1 liter, adjusted to pH 7.0). Leaf cultures were inoculated with 1 ml of a hyphal homogenate containing 2 to 4 mg (dry weight) of hyphal material. Cultures were incubated at 15°C with aeration (0.6 to 0.8 liter of air per min).

Inoculum. Each fungal isolate was grown in shake culture to provide mycelial pellets which were homogenized for the leaf culture inoculum. Flasks (250 ml) containing 35 ml of a yeast extract-glucose broth (NH_4NO_3, 0.80 g; NaCl, 0.11 g; KH_2PO_4, 0.41 g; K_2HPO_4, 0.52 g; $MgSO_4 \cdot 7H_2O$, 0.49 g; $CaCl_2 \cdot 2H_2O$, 0.15 g; glucose, 10 g; yeast extract, 2.0 g; distilled water, 1 liter, adjusted to pH 7.0) were inoculated with 1 ml of a hyphal homogenate prepared by homogenizing a 1% malt extract agar block containing mycelium (0.5 by 2.0 cm) and 5 ml of the medium described above. Cultures were incubated with shaking at 15°C for times previously determined to correspond to the end of the growth phase, e.g., *H. lugdunensis* was incubated for 6 days, *F. curvula*, *A. inflata*, and *T. marchalianum* were incubated for 7 days, *L. aquatica* was incubated for 8 days, and the remaining species were incubated for 10 days. After incubation in yeast extract-glucose broth, mycelial pellets were centrifuged (2,000 × *g*, 15 min), suspended in 30 ml of cold, 0.006 M KH_2PO_4 buffer (pH 7.0), and homogenized in sterile Ten Broeck tissue grinders.

Leaf weight loss. Weight loss due to fungal activities was determined from differences between initial and final dry weights (45°C) of eight tagged leaves per culture. The final dry weight of leaves was determined after the specified incubation times in leaf cultures plus 48 h in stream water at 12°C during feeding experiments.

Invertebrate consumption. Invertebrate consumption was based on the difference between total fractional weight loss of leaves offered to invertebrates and the mean fractional weight loss of leaves from the same culture which were exposed only to fungal activity. Four leaves from each of two replicate cultures of each fungal species were offered in two replicate feeding chambers. Feeding experiments were performed in containers in a constant temperature environment (12°C) for 48 h. The bottoms of feeding chambers were covered with sand and gravel substrata and stocked with fourth and fifth instars of caddisfly larvae of *Hesperophylax* sp. and *Limnephilus* sp.

Penetrometer. Changes in the relative softness of the leaf matrix were estimated with a penetrometer (27).

For each leaf culture, softness was measured as the mean weight required to cause a 5-mm (diameter) rod to penetrate each half of eight leaves held between two Plexiglas plates. Major veins were avoided.

Nitrogen content. Eight dried leaves from each culture were combined and ground in a Wiley mill, and portions were analyzed for total nitrogen with a Carlo Erba Elemental Analyzer.

ATP content. ATP was extracted (14) from two sets of two tagged leaves per culture. Leaves were homogenized (Polytron, setting 7, 15 s) in 5 ml of cold 1.2 N H_2SO_4 containing oxalic acid (8 g/liter) plus 5 ml of 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, Sigma Chemical Co.), pH 7.5. The samples were centrifuged (10,000 × *g*, 20 min) and filtered through glass wool to remove particulates. Aliquots (4 ml) were neutralized with NH_4OH, adjusted to 10 ml, and frozen at −20°C until assayed. Known amounts of ATP were added to replicate aliquots from each fungal treatment to assess efficiency.

Luciferin-luciferase complex (FLE-50, Sigma) was reconstituted with 10 ml of 0.05 M HEPES (pH 7.5) containing 5 mM $MgCl_2$, refrigerated overnight to reduce background luminescence, and centrifuged (10,000 × *g*, 15 min). All enzyme was used the day after it was reconstituted. ATP concentration in aliquots was determined with an integrating photometer (SAI Technology Co.) by comparing the 30-s integration of light emission with a standard curve. Each aliquot was assayed twice.

Mean efficiency for all treatments in the first experiment was 59% (range 42 to 87%); in the second, it was 51% (range 46 to 58%). The values reported here have been corrected with these efficiencies. Mean extraction efficiency for uninoculated leaves in which known amounts of ATP were added before extraction was 56%.

Enzyme assays. Culture fluid was filtered (Whatman no. 50 paper), and thimerosal (Sigma) was added (final concentration, 0.01%) to inhibit microbial growth. Filtrates were then refrigerated until assayed.

Pectin lyase activity was estimated in reaction mixtures containing 1 ml each of 0.2 M bicine with 0.03 M $CaCl_2$ (pH 8.0); 1% pectin (Sigma), dialyzed and centrifuged (9,000 × *g*, 20 min); and the culture filtrate. Reaction mixtures were incubated at 30°C for 3 h and assayed for the production of unsaturated products by reaction with thiobarbituric acid (2). For assay, 0.5 ml of the enzyme reaction mixture was added to 2.5 ml each of 0.1 N HCl and 0.04 M thiobarbituric acid. After 5 ml of water was added, samples were boiled for 30 min, and absorbance was measured at 550 nm (A_{550}). Activity (ΔA_{550} per milliliter per hour) was determined from differences between experimental and control (enzyme added after incubation) assays.

Polygalacturonase, xylanase, and C_x enzymes were estimated in reaction mixtures containing 1 ml each of 0.2 M potassium acetate, pH 5.0; 0.5% of the substrate (polygalacturonic acid, grade II, Sigma; xylan, Sigma, purified fraction V by the procedure of Baker et al. [3]; or carboxymethyl cellulose, Sigma); and the culture filtrate. Reaction mixtures were incubated as described above, and reducing sugars were determined with the dinitrosalicylic acid reagent (9). Activity (nanomoles per milliliter per hour) was determined by comparing absorbance with standard curves prepared by using known concentrations of galacturonic acid, xylose, and glucose, respectively. Control assay values were subtracted from experimental values as described above.

C_1 cellulolytic activity was estimated in assay mixtures containing 1 ml each of 0.2 M potassium acetate and 0.01% thimerosal (pH 5.0), the culture filtrate, and 25 mg of microcrystalline cellulose (Polysciences, Inc.). Reaction mixtures were incubated for 20 h at 30°C. After incubation, reaction mixtures were placed on ice and filtered, and reducing sugars in the filtrate were determined with dinitrosalicylic acid reagent.

Protease activity was estimated in reaction mixtures containing 25 mg of Azocoll (Calbiochem) and 1 ml each of 0.1 M KH_2PO_4 (pH 7.0) and the culture filtrate. Reaction mixtures were incubated at 30°C for 3 h. Incubation was terminated by placing reactions on ice and filtering. The absorbance of the filtrates was measured at 520 nm. Activity (ΔA_{520} per milliliter per hour) was determined from differences between experimental and control assays.

The activities of α-L-arabinofuranosidase, α-D-galactosidase, β-D-galactosidase, β-D-glucosidase, and β-D-xylosidase were estimated by hydrolysis of the respective *p*-nitrophenyl glycosides (Sigma). Reaction mixtures consisted of 1 ml of 0.1 M potassium acetate containing 0.5 mg of the *p*-nitrophenyl glycoside per ml and 0.5 ml each of the culture filtrate and water. Reaction mixtures were incubated at 30°C for 3 h. Reactions were terminated by adding 1 ml of 1 N NH_4OH containing 2 mM disodium-EDTA (16). Samples were diluted (in 3 ml of water), and the absorbance was measured at 400 nm. Activities (nanomoles per milliliter per hour) were determined from the differences between experimental and control assays by comparing absorbances with a standard curve of *p*-nitrophenol.

Statistical analyses. Experiments were analyzed by analysis of variance, and Duncan's multiple range test was used to partition significant treatment effects (23).

RESULTS

Fungal alteration of leaves and invertebrate consumption. Changes in aspen leaves caused by each fungus after growth for 10 days are summarized in Fig. 1. In this experiment, *A. acuminata*, *A. pseudolongissima*, and *L. terrestris* were grown on leaves for 15 days because previous studies indicated that they exhibit slower processing rates than other fungi (Suberkropp and Arsuffi, unpublished data). This allowed differences in processing rates, which can be a factor affecting leaf palatability, to be minimized in the present study. Differential consumption by *Hesperophylax* larvae occurred when larvae were allowed choices among all treatments (Fig. 1, upper panel). Leaves colonized by *A. acuminata* and *F. curvula* were the most palatable. Consumption of leaves of these treatments was significantly different ($P < 0.05$) from each other and from the remaining treatments. Although consumption declined in the sequence, *H. lugdunensis-T. marchalianum-A. pseudolongissima-C. aquatica*, differences between these

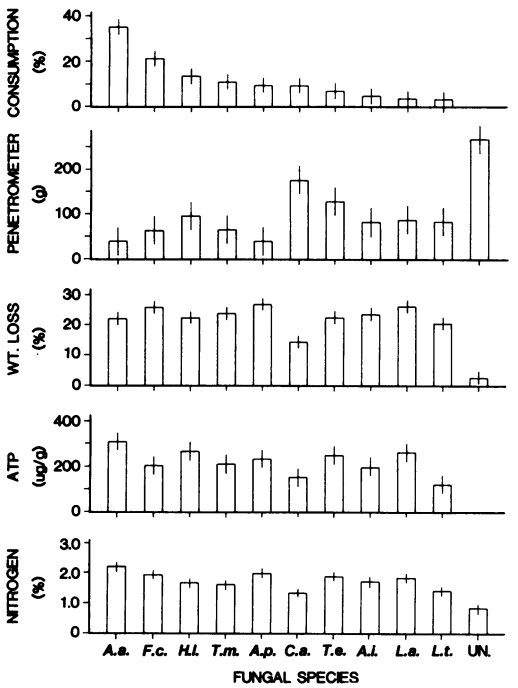

FIG. 1. Feeding preferences and physicochemical changes in aspen leaves incubated with fungi for 10 days. Consumption panel: histograms represent mean fractional consumption by larvae of 16 leaves for each treatment. These leaves were partitioned as four leaves from each of two replicate cultures into each of two replicate feeding chambers. All treatments were offered simultaneously to *Hesperophylax* sp. larvae. Penetrometer and weight loss panels: histograms represent means of two replicate cultures, with measurements made on eight leaves per culture. ATP panel: histograms represent means of two replicate cultures, with measurements made on two sets of two leaves per culture. Nitrogen panel: histograms represent means of two replicate cultures, with three determinations made on pooled and ground samples of eight leaves per culture. Vertical bars on each histogram represent 95% confidence limits from mean square error of analysis of variance. Fungal species abbreviations: *A. a., A. acuminata; F. c., F. curvula; H. l., H. lugdunensis; T. m., T. marchalianum; A. p., A. pseudolongissima; C. a., C. aquatica; T. e., T. elegens; A. i., A. inflata; L. a., L. aquatica; L. t., L. terrestis*; and UN., uninoculated leaves. Cultures of *A. acuminata, A. pseudolongissima*, and *L. terrestis* were incubated for 15 days.

treatments were not significant. Likewise, differences in consumption of the least palatable species (*T. elegens, A. inflata, L. aquatica*, and *L. terrestris*) were not significant.

Physicochemical characteristics of leaves colonized by each fungal species (Fig. 1, lower panels) were generally similar and differed from uninoculated leaves. However, leaves colonized by *C. aquatica* for 10 days in this experiment consistently exhibited less change in all characteristics than was observed for other species. Penetrometer measurements of relative leaf softness and fungus-mediated weight loss among fungal treatments were not significantly correlated with consumption (correlation coefficients, $r = 0.44$ and -0.21, respectively). Fungal biomass (ATP) and total nitrogen concentration of leaves were significantly different ($P < 0.05$) but poorly correlated with consumption ($r = 0.54$ and 0.55, respectively).

To obtain greater resolution of the feeding preference rankings, a second feeding trial was incorporated into this experiment (Fig. 2). In this design, replicate leaves from fungal treatments were divided into three groups which were offered to detritivores in separate feeding chambers. Species composition in each feeding chamber was based on predictions of palatability from previous feeding experiments (Arsuffi and Suberkropp, unpublished data). One set of feeding chambers contained leaves colonized by

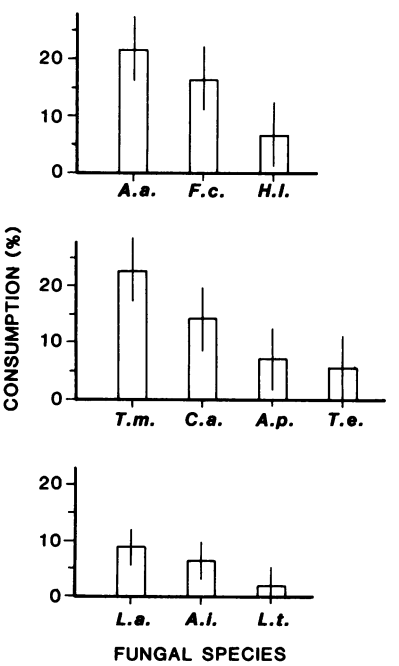

FIG. 2. Feeding preferences of *Hesperophylax* sp. within groups of colonized leaves. Each panel indicates the species composition offered in each set of feeding chambers. Histograms represent the mean fractional consumption of 16 leaves for each treatment. Leaves were partitioned as four leaves from each of two replicate cultures into each of two replicate feeding chambers for each panel. Leaves were from corresponding cultures described in the legend to Fig. 1. Calculation of 95% confidence limits and fungal species abbreviations are as in Fig. 1.

species considered to be most palatable (Fig. 2, upper panel), another set contained leaves colonized by species considered to be intermediate (Fig. 2, middle panel), and the last set contained leaves colonized by the least palatable species (Fig. 2, lower panel). Preference rankings for the first group agreed with results obtained with all species (compare Fig. 2, upper panel, with Fig. 1). When these species were not available, leaves colonized by *T. marchalianum* were consumed significantly more than leaves colonized by *C. aquatica*, *A. pseudolongissima*, and *T. elegens* (Fig. 2, middle panel). When leaves colonized by the three least palatable species comprised the only food source, significant differences in consumption were observed, although leaves colonized by these fungi were not consumed to the same extent as leaves from other combinations (Fig. 2, lower panel).

Changes in aspen leaves caused by each fungus after growth for 20 days are summarized in Fig. 3. Consumption of leaves colonized by different fungi was highly correlated between the two caddisfly genera, *Hesperophylax* sp. and *Limnephilus* sp. ($r = 0.99$; $P < 0.001$). Selective feeding by both caddisfly genera for leaves colonized by *A. acuminata* and *F. curvula* for 20 days was more pronounced (Fig. 3, upper panel) than at 10 days. Leaves colonized by *T. marchalianum* replaced leaves colonized by *H. lugdunensis* as the third most preferred treatment, and consumption of leaves colonized by the remaining six species was minimal. Consumption was not significantly ($P > 0.05$) correlated with penetrometer ($r = -0.37$), fungus-mediated weight loss ($r = -0.21$), ATP ($r = 0.38$), or nitrogen concentration ($r = 0.16$) of the leaves from different fungal treatments.

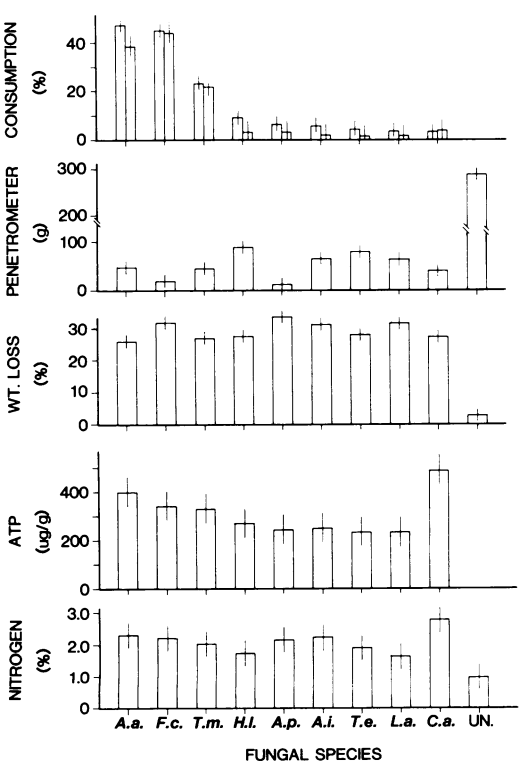

FIG. 3. Feeding preferences and physicochemical changes in aspen leaves incubated with fungi for 20 days. Consumption panel: the first histogram for each fungal species represents the consumption by *Hesperophylax* sp., and the second histogram represents consumption by *Limnephilus* sp. larvae. Replication, 95% confidence limit calculations, and fungal species abbreviations are as in Fig. 1.

Enzyme activity. Activities of polysaccharide-depolymerizing enzymes detected in filtrates of aspen leaf cultures of each fungus are shown in Table 1. All species produced both pectin-degrading enzymes, although pectin lyase activities of *A. pseudolongissima* and polygalacturonase activities of *A. acuminata* and *T. marchalianum* were very low compared with those of other species. Filtrates from all cultures included in the 20-day incubations caused the release of reducing sugars from xylan and carboxymethyl cellulose (C_x) and limited degradation of microcrystalline cellulose (C_1). Of the fungi examined, *H. lugdunensis* exhibited the highest activities for these enzymes (except for polygalacturonase at 20 days) in the culture filtrate.

Only six of the species produced detectable protease activity that acted on Azocoll (Table 2). With the exception of *H. lugdunensis*, activities were generally low. All species elaborated detectable β-D-glucosidase and α-L-arabinosidase, but activities of these enzymes were very low in filtrates of *H. lugdunensis* and *T. marchalianum*, respectively. Most species produced only limited activities of α- and β-D-galactosidases and of β-D-xylosidase. Activities of the galactosidases were noticeably higher in filtrates of *A. pseudolongissima* and *C. aquatica* than in other species. No relationship was observed between the types or activities of any enzyme in the culture filtrates, leaf modification (e.g., softening, ATP), and feeding preferences of caddisfly detritivores for leaves of the different treatments.

DISCUSSION

With respect to our primary objectives, results from the present study lead to two major conclusions. Caddisfly detritivores exhibited selective feeding behavior when offered choices of leaves colonized by the major species of aquatic hyphomycetes occurring in their environment. However, changes in leaves and types of polysaccharidases produced by all species of fungi

TABLE 1. Activities of polysaccharide-depolymerizing enzymes in leaf culture filtrates

Fungal species	Incubation time (days)	Enzyme activity[a]				
		Pectin lyase[b]	Polygalacturonase[c]	Xylanase[c]	C_X[c]	C_1[c]
A. acuminata	15	136 (18)	206 (41)		31 (4)	3 (1)
	20	1,137 (269)	11 (73)	672 (10)	76 (4)	9 (0.5)
F. curvula	10	221 (43)	371 (36)		26 (9)	0
	20	1,077 (73)	1,649 (42)	848 (49)	31 (4)	4 (0.5)
T. marchalianum	10	226 (40)	52 (0)		61 (18)	0
	20	300 (24)	21 (10)	1,511 (19)	111 (4)	17 (0.5)
H. lugdunensis	10	1,072 (246)	1,371 (232)		631 (143)	28 (10)
	20	1,429 (83)	1,019 (95)	2,447 (10)	486 (5)	65 (3)
A. pseudolongissima	15	21 (3)	1,309 (31)		578 (4)	4 (2)
	20	33 (1)	1,770 (97)	1,301 (83)	316 (22)	4 (1)
C. aquatica	10	124 (108)	549 (80)		278 (104)	0
	20	531 (33)	1,339 (100)	1,189 (49)	477 (4)	5 (3)
T. elegens	10	36 (14)	345 (62)		104 (52)	0
	20	142 (0)	704 (63)	1,209 (166)	218 (22)	9 (3)
A. inflata	10	67 (1)	433 (15)		65 (22)	0
	20	294 (2)	1,203 (16)	1,769 (25)	325 (5)	16 (1)
L. aquatica	10	281 (17)	371 (26)		178 (31)	0
	20	546 (8)	630 (31)	1,681 (15)	303 (0)	19 (1)
L. terrestris	15	36 (8)	186 (10)		4 (4)	3 (2)

[a] Numbers in parentheses indicate standard errors of the mean of two cultures.
[b] One unit of activity = a change of 0.001 absorbance unit per ml/h.
[c] One unit of activity = a change of 1 nmol per ml/h.

examined were similar when they were grown with standardized culture conditions. Consequently, selective feeding was not highly correlated with weight loss, softening, ATP content, nitrogen content, or types of enzymes produced by different fungi. Also, several species (e.g., *H. lugdunensis*, *C. aquatica*, *A. pseudolongissima*) became less palatable in comparison with the

TABLE 2. Activities of protease and glycosidases in leaf culture filtrates

Fungal species	Incubation time (days)	Activity[a]					
		Azocoll[b]	β-D-glucosidase[c]	α-L-arabinosidase[c]	α-D-galactosidase[c]	β-D-galactosidase[c]	β-D-xylosidase[c]
A. acuminata	15	0	45 (1)	139 (1)	6 (0)	3 (1)	2 (0.5)
	20	0	51 (2)	109 (14)	6 (0.2)	11 (2)	3 (0)
F. curvula	10	5 (2)	39 (1)	262 (26)	0	6 (1)	2 (0)
	20	213 (50)	72 (6)	255 (11)	3 (1)	31 (3)	2 (0)
T. marchalianum	10	51 (26)	31 (2)	3 (0.5)	0	3 (1)	0
	20	63 (2)	68 (1)	5 (1)	0	8 (1)	0
H. lugdunensis	10	1,524 (668)	7 (0.5)	167 (28)	0	6 (1)	0
	20	430 (20)	3 (0.1)	173 (11)	0	9 (1)	0
A. pseudolongissima	15	91 (5)	163 (14)	34 (1)	119 (1)	68 (4)	10 (1)
	20	298 (22)	133 (10)	34 (2)	320 (24)	63 (12)	6 (1)
C. aquatica	10	21 (5)	40 (1)	59 (14)	6 (2)	129 (44)	2 (0)
	20	295 (20)	80 (14)	484 (8)	66 (1)	1,066 (3)	10 (1)
T. elegens	10	0	44 (5)	661 (57)	6 (3)	13 (3)	3 (0)
	20	0	43 (5)	1,122 (49)	35 (6)	30 (1)	3 (0.5)
A. inflata	10	0	29 (3)	263 (12)	0	3 (1)	2 (0)
	20	0	130 (5)	487 (17)	0	6 (1)	3 (1)
L. aquatica	10	0	55 (1)	316 (14)	0	9 (1)	5 (0.5)
	20	0	173 (1)	245 (6)	0	46 (2)	10 (0.5)
L. terrestris	15	16 (12)	69 (7)	178 (30)	0	3 (1)	5 (0.5)

[a] Numbers in parentheses indicate standard errors of the mean of two cultures.
[b] One unit of activity = a change of 0.001 absorbance unit per ml/h.
[c] One unit of activity = a change of 1 nmol per ml/h.

most preferred species (*A. acuminata, F. curvula*) with increased incubation time (compare Fig. 1 with Fig. 3). This occurred even though levels of ATP, nitrogen, and degradative enzymes did not exhibit dramatic decreases and in some cases increased greatly (e.g., *C. aquatica*). Together, these results suggest that the mechanisms by which caddisfly detritivores select detrital food sources were not related to parameters associated with fungal biomass, degradation of leaves, or capabilities to depolymerize specific polysaccharide components of leaves, as has been suggested (7, 8, 13). Therefore, these results provided evidence to reject our experimental hypothesis, i.e., the parameters associated with fungus-mediated leaf modification measured in this study did not allow adequate prediction of fungal palatability to detritivores.

Although detritivores may obtain increased nutrition from fungal biomass and partial degradation of leaf resources (7, 8, 13), these parameters do not appear to provide the cues that allow caddisfly shredders to discriminate among leaves colonized by different fungi. It is possible that the more palatable species accumulate nutritional factors not directly related to ATP or nitrogen concentrations (e.g., lipids or specific growth factors [13]) or that they are more active in the degradation of inhibitory components in leaf detritus (e.g., lignin or polyphenols). Conversely, unpalatable species may produce distasteful or toxic metabolites (8). Such mechanisms have been demonstrated in plant-herbivore interactions (15, 19) and in similar fashion may represent a means by which these fungi avoid predation by certain detritivores.

Selective feeding on leaf detritus colonized by different fungi under laboratory growth conditions has been demonstrated for amphipod (4) and caddisfly detritivores (present study). Regardless of the mechanism(s) by which detritivores discriminate and selectively feed on leaf material colonized by certain fungi, this capability represents a factor to be considered in understanding species abundances of fungi in streams. In a terrestrial litter system, for example, selective feeding by collembola has been suggested to be an underlying cause for a major shift in the types of fungi observed in the environment (21, 32). Similar potentials appear to be present in stream litter systems, but the impact of selective feeding on fungal populations by detritivores in streams has not been assessed.

With respect to fungal degradative capabilities, five aquatic hyphomycete species (*A. acuminata, C. aquatica, F. curvula, L. aquatica*, and *T. marchalianum*) have been shown to produce enzymes capable of degrading polygalacturonic acid, xylan, and carboxymethyl cellulose (26). These species also caused losses in the cellulose fraction of hickory leaves, indicating that they possess the enzyme complexes necessary to degrade cellulose as it exists in native plant litter. In a study of the pectinases produced by aquatic hyphomycetes, Chamier and Dixon (10) found that all seven species they examined (including two species in common with the present study, *L. aquatica* and *T. elegens*) elaborated polygalacturonases as well as pectin lyase. Results from the present study extend these observations on the degradative enzymology of aquatic hyphomycetes. Of the 10 fungal species examined, all produced each type of polysaccharide-depolymerizing enzyme and at least three types of the glycosidases assayed. Only six species produced protease(s) under these conditions.

The types of polysaccharidases produced by aquatic hyphomycetes are similar to those released into culture filtrates by four species of salt marsh fungi (29, 30) growing on cell wall preparations of cordgrass (*Spartina alterniflora* Loisel). Compared with the salt marsh fungi, a higher percentage of aquatic hyphomycetes produced both types of pectinases, and a lower percentage produced α-D-galactosidase activity. Further characterizations of different fungi and enzymes is needed, but comparisons of these two groups of fungi from different environments suggest that a wide, possibly similar, array of extracellular polysaccharidases is produced by fungi known to be involved in the degradation of plant litter in aquatic systems.

In the characterization of degradative capabilities of fungi growing on plant litter in pure culture, we feel that measurements of specific enzymatic activities are primarily useful in comparing the qualitative potentials expressed by different fungi, i.e., in determining the types of substrates that may be degraded under a particular set of conditions. The magnitude of specific enzymatic activities produced by different fungi, however, does not necessarily reflect the rate at which each species degrades a specific substrate. Certain enzymes produced by different fungi may bind preferentially to plant material (11) or be differentially affected by standardized assay conditions that are not optimum (e.g., in pH, ionic composition, form of substrate) for enzymes of all fungi. Substrates may also be partially masked by polymers for which additional enzymes are required (28). Discrepancies between magnitude of enzymatic activity and rate of degradation of plant cell walls were observed by Torzilli (29) when the filtrates of a fungal species with the highest activities for polysaccharidases did not cause the greatest rate of release of reducing sugars from cell wall preparations. Similar results were noticed in the present study for *H. lugdunensis*, which exhibit-

ed the highest activities for most of the enzymes assayed yet caused less leaf softening and weight loss than several other species.

Comparison of the physicochemical changes in leaves (Fig. 1 and 3) as well as the types of extracellular enzymes (Tables 1 and 2) produced by the aquatic hyphomycetes examined in the present study indicates that all species possess similar degradative capabilities under these conditions. These results suggest that a high degree of overlap exists among these fungi with respect to resource utilization. In addition, all species (except for *T. elegens*) were found to coexist in the same stream habitat (Suberkropp and Arsuffi; unpublished data). These observations lead us to conclude that abundances and reproductive success of these fungi may be more closely related with differential responses to abiotic and biotic factors (e.g., temperature, type of leaf, invertebrate predation) than to differences in degradative capabilities. Clearly, further characterization of such interactions is required to improve our understanding of resource utilization and partitioning by these fungi.

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