Degradation of lignocellulose in wheat straw versus hardwood by Cyathus and related species (Nidulariaceae)*

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Twelve species of Cyathus and two additional taxa included in the Nidulariaceae (i.e., Crucibulum laeve (Bull. ex DC) Kambly and Nidula niveo-tomentosa (Henn.) Lloyd) were examined for their ability to differentially modify the lignin and cellulose components of wheat (Triticum aestivum L.) straw (WS) and a silver maple (Acer saccharinum L.) hardwood (HW) log. Our results indicate that the rate and pattern of biological modification of native lignocellulose differs according to substrate and fungal species chosen for the fermentation. The ecological specialization or substrate preferences of the species selected for study was not always an accurate predictor of how each fungus ranked in its ability to degrade WS or HW.


Douze espèces de Cyathus et deux taxons additionnels inclus dans les Nidulariaceés (c.-à-d. Crucibulum laeve (Bill. ex DC) Kambly et Nidula nivea-tomentosa (Henn.) Lloyd) ont été étudiées quant à leur aptitude à modifier de façon différentielle les composants cellulose et lignine de la paille (WS) de blé (Triticum aestivum L.) et de billes de bois dur (HW) d’érable argenté (Acer saccharinum L.). Les résultats indiquent que les taux et les modes de modification biologique des sources lignocellulosiques diffèrent selon les substrats et les espèces fongiques choisies pour la fermentation. La spécialisation écologique ou les préférences des substrats des espèces choisies pour l’étude n’ont pas toujours été des indices de prédiction précis quant au rang d’aptitude de chaque champignon pour la dégradation des WS ou HW.

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The bird’s nest fungi (Nidulariaceae) are basidiomycetes that colonize wood (often in the form of small twigs or branches), dead herbaceous stems, dung of horses and cows, and plant fiber products. Given such substrate preferences, one would expect these fungi to be capable of biologically degrading the cohesive lignocellulosic complex in plant cell walls. Included in the Nidulariaceae is the genus Cyathus, which encompasses a tightly circumscribed group of 43 recognized species (Brodie 1975). Using a strain of Cyathus stercoreus (Schw.) de Toni NRRL 6473 isolated from cattle dung, Wicklow, Detroy, and Jessee (1980) and Abbott et al. (1983) have demonstrated that the fungus effected substantial losses in lignin from wheat straw during fermentation. The basidiomycete also improved wheat straw digestibility by freeing α-cellulose for enzymatic hydrolysis to glucose. The rationale for selecting Cy. stercoreus in attempting to biologically modify the lignin and cellulose of wheat straw or other gramineous agricultural residues was based on the expectation that this organism is ecologically specialized to attack enzymatically the substructures of native lignins in grasses. Central to this reasoning are the facts that natural lignins of grasses are known to differ from those of other plant taxa in the relative frequency of certain chemical substructures (Kirk 1971; Kirk et al. 1975; Sarkanen and Ludwig 1971) and that undigested lignocellulose from grasses is a principal component of cattle dung in grasslands. Is Cy. stercoreus coprophilous because it has limited ability to degrade lignocellulose from hardwoods? Are species of Cyathus from hardwoods less efficient in attacking the lignocellulose of grasses? In the present study we examined the differential modification of the lignin and cellulose components of wheat straw (Triticum aestivum L.) and a silver maple (Acer saccharinum L.) log by 12 species of Cyathus and two additional taxa included in the Nidulariaceae (Crucibulum laeve (Bill. ex. DC) Kambly and Nidula niveo-tomentosa (Henn.) Lloyd).

Strains of bird’s nest fungi (Nidulariaceae) selected for study are listed below, with specific information as to the source of each strain and a brief summary of substrate—habitat and biogeographic distribution records for individual taxa: Cyathus africanaus Brodie NRRL 6519 (=Brodie 66120) on prunings of Cupressus from Mount Kilimanjaro, Tanzania, no other records; Cyathus berkeleyanus (Tul.) Lloyd NRRL 6520 (=Brodie 6694) on old wood. Trois Rivières, Guadeloupe, widespread in the New World tropics; Cyathus bulleri Brodie NRRL 6521 (=Brodie 6680a) on old wood. Trois Rivières, Guadeloupe, known from New World tropics; Cyathus canna Lloyd NRRL 6522 (=Brodie 1238) on sawdust, Costa Rica.

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the United States Department of Agriculture over other firms or similar products not mentioned.
known from New World tropics; **Cyathus earlei** Lloyd NRRL 6523 (= Brodie 1286) on soil at edge of potato field, Vera Cruz, Mexico, known from New World tropics and subtropics; **Cyathus helenae** Brodie NRRL 6524 (= Brodie 1500) on old stems Rocky Mountain Park, Alberta, Canada, no other records; **Cyathus julietae** Brodie NRRL 6526 (= Brodie 6641) on old wood, Hardwar Cap, Jamaica, no other records; **Cyathus limbatus** Tul. NRRL 6527 (= Brodie 6688) on old wood, Goyave, Guadeloupe, worldwide in tropics and subtropics; **Cyathus pygmaeus** Lloyd NRRL 6530 (= Brodie 66133) on old stems of *Artemisia*, Owyhee County, Idaho, from arid regions of northwestern U.S.A.; **Cyathus sertoreus** (Schw.) de Toni NRRL 6473 (= Brodie 6661) on soil at edge of potato ferreling one 5-mm block of agar, removed from the margin of a 6-day-old actively growing colony, to each flask before autoclaving 50 min at 15 psi (1 psi = 6.895 kPa) to sterilize the flask contents.

Fermented substrates were dried to constant weight in a forced-air drying oven at 80°C, and biomass losses were determined by calculating differences in the final dry weight (dw) compared with the initial weight before fermentation. Dried samples were ground with a Tecator Cyclotec sample mill (Tecator Inc., Boulder, CO) equipped with a 1-mm screen. The milled samples were used in all subsequent substrate analyses. Cellulose and lignin content were determined by the permanganate—lignin method of Goering and Van Soest (1970) using Tecator Fibertec System extraction units (Tecator Inc., Boulder, CO).

To determine the digestibility of fermented residues in the laboratory, 1000 mg of milled sample, 4.0 mL of stock cellulase enzyme solution, and 16 mL buffer were added to a 50-mL Erlenmeyer reaction flask. Stock enzyme solution (*Trichoderma viride*, 0.37 FPU/mg concentrate) consisted of 20 mg cellulase powder (assay No. 27,125 obtained from Miles Laboratories, Elkhart, IN) in pH 4.6 citric acid—sodium phosphate buffer solution. Reaction flasks containing sample and enzyme were tightly sealed with rubber stoppers and incubated in a water bath at 55°C with shaking (70 pulses/min) for 6 h. When the digestion was completed, clear filtrates were obtained by gravity filtration through Whatman No. 4 filter paper. Carbohydrates were analyzed by high-pressure liquid chromatography (HPLC) on a Waters HPLC fitted with a DuPont Zorbax-NH3 column. The mobile phase consisted of acetonitrile—H2O (70:30) using refractive index as a monitor. A background glucose level of 1.1 mg per 4 mL of Miles cellulase preparation was subtracted from the total glucose content of each digest. The following formula was used to calculate conversion (percent) of cellulose in fermented residue to glucose:

\[
\text{percent conversion} = \frac{\text{milligrams of glucose (162/180)}}{\text{milligrams of cellulose (dw)}} \times 100
\]

The factor 162/180 normalizes the conversion for the weight gain owing to addition of water to the glucosyl moiety on hydrolysis. Correlation coefficients based on fungal growth rate (millimetres per day) over the surface of PDA and substrate decomposition (Table 1) were nonsignificant at the 5% level for both WS \((r = 0.4999)\) and HW \((r = 0.1919)\). Neither Garrett (1975) nor Wicklow, Detroy,
<table>
<thead>
<tr>
<th>Taxa</th>
<th>Growth rate (mm/day)*</th>
<th>Weight loss, dw (%)</th>
<th>Cellulose remaining (%)</th>
<th>Lignin remaining (%)</th>
<th>α-Cellulose content (mg) of 1000-mg fermented sample</th>
<th>Glucose (mg) from 1000-mg sample by enzymatic hydrolysis</th>
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<tr>
<td>Control</td>
<td>NA</td>
<td>WS = 1.6</td>
<td>WS = 100</td>
<td>WS = 352</td>
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<td>26(6)</td>
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<td>12.5</td>
<td>87</td>
<td>86</td>
<td>368</td>
<td>70(17)</td>
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<td>7</td>
<td>25</td>
<td>75</td>
<td>75</td>
<td>354</td>
<td>161(41)</td>
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<td><em>Cyathus bulleri</em> Brodie NRRL 6521</td>
<td>8</td>
<td>15.6</td>
<td>84</td>
<td>83</td>
<td>370</td>
<td>102(25)</td>
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<tr>
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<td>5.9</td>
<td>17.2</td>
<td>81</td>
<td>84</td>
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<td>6.5</td>
<td>18.9</td>
<td>81</td>
<td>80</td>
<td>364</td>
<td>160(40)</td>
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<td><em>Cyathus julietae</em> Brodie NRRL 6526</td>
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<td>18.8</td>
<td>80</td>
<td>82</td>
<td>374</td>
<td>147(35)</td>
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<td><em>Cyathus limbatus</em> Tul. NRRL 6527</td>
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<td>12.2</td>
<td>88</td>
<td>84</td>
<td>356</td>
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<td><em>Cyathus pallidus</em> Berk. &amp; Curt. NRRL 6529</td>
<td>9</td>
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<td>82</td>
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<td><em>Cyathus pygmaeus</em> Lloyd NRRL 6530</td>
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<td>7.3</td>
<td>93</td>
<td>91</td>
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<td>45(11)</td>
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<td><em>Cyathus striatus</em> (Huds.) Willd. ex Pers. NRRL 6532</td>
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<td>83</td>
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<td><em>Crucibulum laeve</em> (Bull. ex DC) Karnblzy NRRL 6518</td>
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<td>89</td>
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<td>40(10)</td>
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<td><em>Nidula niveo-tomentosa</em> (Henn.) Lloyd NRRL 6533</td>
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<td>10.1</td>
<td>90</td>
<td>88</td>
<td>367</td>
<td>439</td>
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</table>

*Growth rate estimated by dividing the average colony diameter (three colonies by the number of days (at 60 mm or at 8 days) for which colony growth on individual plates of PDA was recorded.
*Calculations were based upon two independent growth experiments with triplicate analysis per 45-day growth experiment.
*Analyses were made by Goering – Van Soest method for cellulose content (Goering and Van Soest 1970).
*Lignin values were determined by the permanganate lignin method (Goering and Van Soest 1970).
*Hydrolysis of 1000 mg of straw sample with 10 IU of cellulase per gram of residue for 4 h. One international unit equals 1 μmol produced as glucose from filter paper per minute (Gong et al. 1977). Enzyme was obtained from Miles laboratories, Elkhart, IN. Glucose (milligrams) was determined by high-pressure liquid chromatography.
*NA, not applicable.
*The values in parentheses represent the percent conversion of cellulose to glucose. Conversion data were adjusted for weight gain from water addition to glucosyl moiety on hydrolysis. Formula = percent conversion = ((grams of glucose produced) (162/180) (100)) / grams of cellulose (dw).
tive than *Cy. stercorulis* in increasing the amount of available carbohydrate as measured by calculating the percentage conversion of the remaining cellulose to glucose (Table 1, Fig. 1). These results indicate that while *Cy. stercorulis* can efficiently attack the lignocellulose in WS, it also utilizes a greater portion of the cellulose fraction, as contrasted with *Cy. berkeleyanus*, *Cy. canna*, *Cy. helenae*, *Cy. jullietae*, and *Cy. pallidus*, all of which colonize old wood. *Cyathus canna* was the most effective organism in the biological modification of lignocellulose to render the cellulose in WS more susceptible to hydrolysis by cellulase (47% conversion of cellulose to glucose). Conversion of cellulose to glucose by *Cy. canna* was four times that of the unfermented WS control. Four of the fungi tested did not enhance the digestibility of WS (e.g., *Cy. limbatus*, *Cy. pygmaeus*, Crucibulum laeve, and Nidula niveo-tomentosa).

None of the fungal strains tested were able to effect the same biomass losses in HW that were recorded for WS (Table 1). *Cyathus berkeleyanus* brought about the greatest weight loss in HW; however, other species (e.g., *Cy. canna*, *Cy. helenae*, *Cy. limbatus*, *Cy. pallidus*, and *N. niveo-tomentosa*) were better able to improve the digestibility of HW resulting from an increase in the concentration of available cellulose. Our isolate of *Cy. stercorulis* from old cow dung was one of the four most active degraders of HW lignocellulose (10% weight loss), but its cellulose-to-glucose conversion rate (11%) ranked ninth among the 14 species tested (Table 1, Fig. 2).

### Fig. 1
Decline in percent cellulose or percent lignin with increasing substrate weight loss for (a, b) wheat straw and (c, d) hardwood.

### Fig. 2
Percent conversion of cellulose to glucose in chopped wheat straw versus hardwood shavings following fermentation.

and Adams (1980) found a correlation between the ability of the fungus to grow rapidly over the surface of PDA and the rate at which that fungus could degrade WS.

The results indicate that decomposition of WS or HW by species of Nidulariaceae, as measured by weight loss, is positively correlated with degradation losses of both cellulose and lignin (Table 1). *Cyathus berkeleyanus*, isolated from old wood, and *Cy. stercorulis*, from old cow dung, effected the greatest biomass losses and were also the most efficient in degrading the lignin and cellulose components of WS (Table 1). However, *Cy. berkeleyanus* was more effective than *Cy. stercorulis* in increasing the amount of available carbohydrate as measured by calculating the percentage conversion of the remaining cellulose to glucose (Table 1, Fig. 1). These results indicate that while *Cy. stercorulis* can efficiently attack the lignocellulose in WS, it also utilizes a greater portion of the cellulose fraction, as contrasted with *Cy. berkeleyanus*, *Cy. canna*, *Cy. helenae*, *Cy. jullietae*, and *Cy. pallidus*, all of which colonize old wood. *Cyathus canna* was the most effective organism in the biological modification of lignocellulose to render the cellulose in WS more susceptible to hydrolysis by cellulase (47% conversion of cellulose to glucose). Conversion of cellulose to glucose by *Cy. canna* was four times that of the unfermented WS control. Four of the fungi tested did not enhance the digestibility of WS (e.g., *Cy. limbatus*, *Cy. pygmaeus*, Crucibulum laeve, and Nidula niveo-tomentosa).

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Natural lignins differ in the relative frequency of certain chemical substructures, and these differences are especially great between angiosperms and gymnosperms and between different species of angiosperms (Kirk 1971; Sarkanen and Ludwig 1971; Kirk et al. 1975). Our results indicate that the rate and pattern of biological modification of native lignocellulose in a gramineous straw and a hardwood log differ according to both substrate type and fungal species chosen for the fermentation. Species that were among the most effec-
tive in attacking wheat straw were not necessarily the most active in attacking the native lignocellulose in the hardwood and vice versa.

The enzymes responsible for lignin degradation have not been identified. There is evidence to suggest that the degrading system is quite nonspecific and that enzymatically generated oxidizing species may be the actual agents responsible for the oxidative reactions (Bar-Lev and Kirk 1981). If lignin biodegradation is brought about through a mechanism of random oxidation, then the differing abilities of Cyathus species to degrade the lignin in WS or HW lignocellulosic complexes must be related to other genetic or environmental factors affecting fungal growth on those substrates or to fungal ability to enzymatically generate oxidizing species. It is not surprising then that the ecological specialization or substrate preference of the species of Nidulariaceae selected for study (Brodie 1975) was not always an accurate predictor of how these fungi ranked in their ability to degrade WS or HW in a laboratory fermentation environment.

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