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Evaluation of the efficacy of the photosystem II inhibitor DCMU in periphyton and its effects on nontarget microorganisms and extracellular enzymatic reactions

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Abstract. We examined the efficacy of the photosystem II inhibitor 3-(3,4-diclorophenyl)-1,1-dimethyl urea (DCMU) for inhibition of algal photosynthesis in periphyton associated with submerged decomposing litter of *Typha angustifolia*. We also investigated the possible nontarget effects of DCMU exposure on heterotrophic microorganisms (i.e., bacteria and fungi) and extracellular enzyme activity associated with decaying litter. Standing-dead *Typha* leaf litter was submerged for 34 and 73 d, returned to the laboratory, and used for controlled laboratory experiments that examined the effect of DCMU on algal ([^{14}C]bicarbonate, pulse-amplitude modulated fluorometry), bacterial ([^{3}H]leucine), and fungal ([^{14}C]acetate) production. Simultaneous assays also were conducted to examine the effect of DCMU on the activities of 4 extracellular enzymes (β-glucosidase, β-xylosidase, leucine-aminopeptidase, and phosphatase). DCMU significantly inhibited algal photosynthesis in light-exposed periphyton (p always < 0.0003), with strong inhibitory effects occurring within 5 min after exposure to DCMU. In contrast, DCMU had no significant direct effect on bacterial ($p >$ 0.5) or fungal production ($p > 0.3$). Extracellular enzyme activities also were not significantly affected by exposure to DCMU. Heterotrophic microbial and enzyme activity assays were conducted in darkness to avoid any indirect effects of DCMU (i.e., heterotrophic responses to the inhibition of photosynthesis, rather than to DCMU itself). The apparent lack of nontarget effects of DCMU on heterotrophic microbial processes, combined with good efficacy against algal photosynthesis, suggest that DCMU may a useful selective inhibitor for investigations of interactions among litter-inhabiting microbiota.

Key words: wetlands, photosynthesis, production, algae, fungi, bacteria, extracellular enzymes.

Periphyton is the assemblage of microorganisms (i.e., algae, bacteria, fungi, protists) that colonize and grow on surfaces within aquatic ecosystems (Wetzel 2001). These communities often form thick biofilms on substrata, and in the case of fungal organisms colonizing detritus, also grow endogenously (i.e., hyphal penetration) within the substratum itself. In aquatic ecosystems exposed to sunlight, the photosynthetic activities of periphytic algae often can contribute a substantial portion of the ecosystem's net primary production, and much of this primary production can be passed to higher trophic levels (e.g., Stevenson 1996, Wetzel 2001). Heterotrophic microbial assemblages, particularly those communities growing upon and within plant litter, also might support the nutritional requirements of consumers, thus forming another link to higher trophic levels of aquatic food webs (e.g., Cummins 1974, Bärlocher 1985, Graça et al. 1993, Hall and Meyer 1998). In addition, as a result of both intense nutrient uptake and substantial nutrient mineralization and release, periphyton communities can strongly influence nutrient cycling in aquatic ecosystems by acting as either a nutrient source or sink (Mulholland 1996, Wetzel 1996, 2001). As a consequence, periphyton communities play an important role in the functioning of aquatic ecosystems.

Periphytic microbiota interact with one another. For example, heterotrophic microbes (i.e., bacteria) and extracellular enzyme activity within periphyton can be

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strongly affected by algal photosynthesis (Murray et al. 1986, Neely and Wetzel 1995, Espeland et al. 2001, Francoeur and Wetzel 2003, Francoeur et al. 2006). Algal photosynthesis can stimulate nitrification in periphyton (Lorenzen et al. 1998, An and Joyce 2001, Kemp and Dodds 2001), but it is inhibitory to periphytic bacterial and cyanobacterial N_2 fixation (Bebout et al. 1987, 1993, Paerl et al. 1996) and bacterial denitrification (Christensen et al. 1990, Nielsen et al. 1990).

Previous studies assessing the interactions among microbes (e.g., DeLorenzo et al. 2001), or participation of specific microbial groups in ecological processes (Mason 1976, Padgett et al. 1985, Hackney et al. 2000) in natural periphytic or detrital samples often have used selective inhibitors to disrupt the growth of certain microbial groups (e.g., bacteria or fungi). Various broad-spectrum growth inhibitors targeting prokaryotes (e.g., streptomycin) or eukaryotes (e.g., cycloheximide) often are used, despite well-known methodological shortcomings, such as inhibitor resistance by some taxa or nontarget organism effects, when used in mixed assemblage systems (Oremland and Capone 1988).

The simple method of placing communities in ambient light or complete darkness often is used to regulate photosynthetic activity in periphyton (e.g., Biggs et al. 2000, Espeland and Wetzel 2001b, Espeland et al. 2001, Francoeur and Wetzel 2003, Francoeur et al. 2006). However, manipulations of light and dark treatments are not without drawbacks. Aside from the occasional difficulty and inconvenience of ensuring complete darkness within dark treatments, important parameters dependent upon light exposure, such as temperature, photorespiration (Wetzel 2001), ultraviolet (UV) photolysis of recalcitrant dissolved organic C (DOC) to more labile compounds (Wetzel et al. 1995, Wetzel 2000), inactivation of extracellular enzymes via UV photolysis (Espeland and Wetzel 2001a), and light-mediated uptake of labile DOC (Paerl et al. 1993), potentially could be biased by light manipulation.

The photosystem II inhibitor 3-(3,4-diclorophenyl)-1,1-dimethyl urea (DCMU) has been used in place of light manipulation to control photosynthetic activity in periphyton (Paerl et al. 1993, Neely and Wetzel 1995, Kahn and Wetzel 1999, Staats et al. 2000, DeLorenzo et al. 2001, Espeland and Wetzel 2001b). Few studies have quantified the efficacy of DCMU or the time required for DCMU exposure to halt photosynthetic activity; times ranging from <5 min (Paerl et al. 1993, Neely and Wetzel 1995) to >1 d (Espeland and Wetzel 2001b) have been reported. Little is known about the potential direct effects of DCMU on nontarget microorganisms and processes. Neely and Wetzel (1995) found no significant direct effect of DCMU on the productivity of wetland heterotrophic bacteria. In most other instances, the direct effects of DCMU on nontarget microorganisms and processes simply have been assumed to be nonexistent. The purpose of our study was to: 1) determine the efficacy of DCMU exposure for halting algal photosynthesis in periphyton attached to wetland plant detritus, and 2) investigate possible direct effects of DCMU exposure on nontarget heterotrophic microorganisms (i.e., bacteria, fungi) and extracellular enzyme activity.

Methods

Microbial community generation and field sampling

Standing dead *Typha angustifolia* leaf litter was collected from the Paint Creek wetland (lat 42°12.971′N, long 83°37.181′W), returned to the laboratory, air-dried, and stored at ambient laboratory temperatures until used. Dried litter was cut into ~16-cm-long sections and attached to floating wire-mesh trays with silicone sealant at the ends (~1–2 cm) of each litter section. A metal strip was attached to the sealant to clamp litter pieces in place. Mesh trays were submersed in the wetland (~1–2 cm in depth) on 8 April 2005 and retrieved after 34 d (experiment 1: microbial production only) or 73 d (experiment 2: microbial production, photosystem II activity, and extracellular enzyme activity). Litter sections were removed from trays by carefully cutting and gently removing the middle section (~12 cm) of each piece from the ends attached to the tray. Litter sections were gently enclosed in resealable containers with wetland water, placed on ice in a cooler, and returned to the laboratory (<30 min). In the laboratory, litter was cut into either 1.7-cm-long pieces (biomass and production assays, ~3.22 cm^2 total surface area) or 0.85-cm-long pieces (enzyme activity assays, ~1.61 cm^2 total leaf surface area).

Concurrent with microbial sample collection, surface-water samples were collected in acid-washed bottles, placed on ice, and returned to the laboratory for analysis of pH, alkalinity, and concentrations of total P, soluble reactive P (SRP), NH_4^+, and NO_3^-+NO_2^-. Surface-water pH was measured using a Mettler-Toledo MP220 bench-top pH meter (Mettler-Toledo, Columbus, Ohio). Alkalinity was determined by titration to pH 4.5 (APHA 1992). Samples for SRP, NH_4^+ and NO_3^-+NO_2^- were filtered (0.2-μm pore size) and then frozen (–10°C) until analysis; samples for total phosphorus (TP) were frozen without filtration. All N and P concentrations were measured using a Lachat AP300 discrete analyzer (Lachat, Milwaukee, Wisconsin). TP concentrations were assayed using a persulfate digestion and the molybdate/ascorbic acid method as outlined in Hebert and Green (2005). SRP

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was determined using the molybdate/ascorbic acid method in Hebert (2005). NH_4^+ was assayed using the phenol/nitroprusside/hypochlorite method for unpreserved samples described in Hebert (2004). NO_3^-+NO_2^- concentrations were determined using the Cd reduction method in Harbridge (2005).

Analysis of microbial biomass, microbial composition, and litter mass

Algal biomass associated with litter was measured as chlorophyll *a*, algal community composition was determined using brightfield microscopy, bacterial biomass associated with litter was determined by epifluorescence microscopy, and fungal biomass was estimated from concentrations of ergosterol in/on plant litter. These analyses followed the protocols detailed in Francoeur et al. (2006). Total litter ash-free dry mass (i.e., microbial + plant litter organic mass) was measured by drying (105°C, 24 h), weighing, combusting (500°C, 4 h), cooling (in a desiccator), and reweighing 4 to 5 replicate samples from each experiment. Total organic C was assumed to be 50% of total ash-free dry mass.

Effects of DCMU on microbial production and extracellular enzyme activity

A 2000 μM stock solution of DCMU (Sigma-Aldrich, St. Louis, Missouri) was prepared in acetone. For each experiment, this DCMU stock solution was added to wetland water to make working solutions of 20 μM DCMU. Equivalent amounts of pure acetone were added to DCMU-free wetland water to control for any effect of acetone. The final acetone concentration was low (0.01% v/v), and any acetone effects on microbial production and enzyme activity were assumed to be negligible. Bergman (1980) and Lopez-Rodas et al. (2001) used similar protocols with ethanol or dimethyl sulfoxide as the organic solvent for DCMU.

Rates of [^{14}C] bicarbonate incorporation into attached algae (method of Francoeur et al. 2006) were monitored to quantify the effects of DCMU exposure on periphytic algal photosynthesis. Litter sections were incubated (2 h, 20°C, 400 μmol m^{-2} s^{-1} photosynthetically active radiation [PAR]) in filtered wetland water with 0.5 μCi of $H[^{14}C]O_3^-$. In each experiment, 3 replicates were spiked with 20 μM DCMU, whereas 3 other replicates remained DCMU free. Killed controls (3% v/v formalin) were used to correct for nonbiological [^{14}C] incorporation. Inorganic C pools were estimated by measuring alkalinity (titration to pH 4.5; APHA 1992). After incubation, litter and attached algae were killed with 3% formalin (final concentration) and filtered, and litter and filters were stored frozen (−20°C). Samples were later fumed with HCl and dissolved in NaOH. Aliquots were then cleared with 50% H_2O_2, mixed with scintillation fluid, and they were assayed for radioactivity (Beckman LS 6500 Scintillation Counter, corrected for quenching; Beckman Coulter, Fullerton, California). Production was calculated using the equations of Wetzel and Likens (2000).

To investigate the rapidity of DCMU inhibition of photosynthesis, pulse-amplitude modulated (PAM) fluorometry was used to measure periphytic algal photosystem II electron transport rates after DCMU exposure during experiment 2. Three litter sections were placed in wetland water spiked with 20 μM DCMU, and an additional 3 sections were placed in DCMU-free wetland water. Light-adapted photosystem II yield (i.e., the Genty parameter, $\Delta F/F'_m$) (Genty et al. 1989, Kromkamp and Forster 2003) was measured every 5 min for 15 min using a stand-alone PAM fluorometer (Diving-PAM, Heinz Walz GmbH, Effeltrich, Germany). Samples were held in uniform, low-light conditions ($\leq$15 μmol m^{-2} s^{-1} PAR) on the laboratory bench before and during experimentation.

Rates of [^{3}H] leucine incorporation into bacterial protein (method of Gillies et al. 2006) were used to quantify the effects of DCMU exposure on periphytic bacterial production. Litter sections were incubated (30 min, 20°C, in complete darkness, 0 μmol m^{-2} s^{-1} PAR) in sterile vials with filtered (0.22-μm pore size) wetland water containing 2500 nM leucine (specific activity = 220 GBq/mmol). In each experiment, 5 vials were spiked with 20 μM DCMU, whereas 5 other vials remained DCMU free. Killed controls (5% v/v trichloroacetic acid [TCA]) were used to correct for nonbiological [^{3}H] leucine incorporation. Incubations were terminated with 5% (final concentration) TCA, and then heated (80°C, 30 min). After cooling on ice for 30 min, samples were filtered (0.22-μm pore size), rinsed with ice-cold solutions of 5% TCA (3×), 80% ethanol (2×), and double-distilled H_2O (2×); dissolved in alkaline extractant (0.3 M NaOH, 0.1% sodium dodecyl sulfate [SDS], 25 mM ethylenediamine tetraacetic acid [EDTA]); and heated (80°C, 60 min). After cooling, aliquots were neutralized with HCl, dialyzed against ammonium bicarbonate buffer (0.2 M NH_4HCO_3, 0.1 M NaCl, 0.1% SDS, 25 mM EDTA; 500 molecular weight cut-off dialysis membrane), cleared with 50% H_2O_2, mixed with scintillation fluid, and assayed for radioactivity (Beckman LS 6500 Scintillation Counter, corrected for quenching).

Effects of DCMU on instantaneous growth rates of fungi associated with plant litter were determined by quantifying rates of [^{14}C] acetate incorporation into ergosterol (Gessner and Newell 2002). Litter sections

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were incubated (5 h, 20°C, in complete darkness, 0 μmol m^{-2} s^{-1} PAR) in sterile vials containing filtered (0.22-μm pore size) wetland water and 5 mM Na[1-^{14}C] acetate (specific activity = 37 MBq/mmol). In each experiment, 5 vials were spiked with 20 μM DCMU, whereas 5 other vials remained DCMU free. Killed controls (2% v/v formalin) were used to correct for nonbiological [^{14}C] acetate incorporation. Incorporation of [^{14}C] acetate label was stopped by placing vials on ice and immediately filtering (1.2-μm pore size) the contents. Filters and litter pieces were washed twice with filtered (0.7-μm pore size) wetland water and stored at −20°C. Ergosterol was extracted and analyzed as described previously (Francoeur et al. 2006). Ergosterol fractions eluted from the high-performance liquid chromatography (HPLC) column were collected, mixed with scintillation fluid, and assayed for radioactivity (Beckman LS 6500 Scintillation Counter, corrected for quenching). The [^{14}C] acetate incorporation rates were converted to fungal growth rates assuming 12.6 μg of fungal biomass/nmol acetate incorporated (Gessner and Newell 2002).

Incubations of litter sections with the appropriate fluorogenic substrate (4-methylumbelliferyl β-D-glucopyranoside, 4-methylumbelliferyl β-D-xylopyranoside, L-leucine 7-amido-4-methylcoumarin hydrochloride, 4-methylumbelliferyl phosphate disodium salt, 4-methylumbelliferyl α-D-glucopyranoside) dissolved in autoclaved wetland water were used to investigate the effects of DCMU exposure on the activities of periphytic β-glucosidase, β-xylosidase, leucine-aminopeptidase, and extracellular phosphatase, respectively. Before enzyme activity assays, litter sections were placed in Petri dishes with either 20 μM DCMU-spiked wetland water or DCMU-free wetland water for ≥5 min to allow DCMU exposure to inhibit photosynthetic activity. After this preincubation period, enzyme activities were quantified using the method of Francoeur et al. (2006). Litter sections were incubated (30 min, 20°C, in complete darkness, 0 μmol m^{-2} s^{-1} PAR) in sterile glass vials with a saturating amount of substrate. For each enzyme assayed, 5 vials were spiked with 20 μM DCMU, whereas 5 other vials remained DCMU free. Litter-free vials served as controls for nonenzymatically produced fluorescence. After incubation, an aliquot from each sample was immediately added to pH 10 carbonate/bicarbonate buffer (pHydrion, Micro Essential Laboratory, Brooklyn, New York) in individual wells of a black 96-well plate, and fluorescence was measured without delay using a Fluoroskan Ascent plate reader (excitation wavelengths: 355 ± 40 nm, emission wavelengths: 460 ± 40 nm). Methlyumbelliferone sodium salt and 7-amino-4-methylcoumarin standards were included on all plates to allow conversion of raw fluorescence to concentrations. Reaction velocities were determined by dividing the amount of substrate hydrolyzed by total litter C and incubation time.

Data analysis

The hypothesis that DCMU exposure would inhibit algal productivity was tested by: 1) comparing observed algal production (based on ^{14}C incorporation) in the presence of DCMU to 0 with 1-sample *t*-tests, 2) comparing observed algal production in the presence and absence of DCMU with 2-sample *t*-tests, and 3) comparing fluorescence-based photosynthetic yield measurements in the presence and absence of DCMU with 2-sample *t*-tests. The hypotheses that DCMU exposure would affect bacterial production, fungal production, and extracellular enzyme activity were tested by comparing the relevant response variable in the presence and absence of DCMU with 2-sample *t*-tests. All *t*-tests used 2-sided alternative hypotheses, and all 2-sample *t*-tests considered independent variances. A power analysis was done for each 2-sample *t*-test of microbial production or enzyme activity. The power of each existing experiment was calculated based on the assumption that the observed sample means and pooled standard deviations (method of Cohen 1977) of each experimental treatment were true values of population means and standard deviations, and the number of replicates needed to achieve a power of 0.90 (at $\alpha = 0.05$) for rejecting a 2-sided null hypothesis was calculated. All statistical calculations were done using SYSTAT (version 10.2; Systat Software, San Jose, California).

Results

Microbial communities and environmental conditions

The pH (7.85 ± 0.23) and alkalinity (243.5 ± 31.8 mg $CaCO_3$/L) of wetland surface waters during the study were consistent with values previously observed in the Paint Creek wetland (Francoeur et al. 2006). Nutrient levels (mean ± 1 SD; 151.5 ± 164.8 μg TP/L, 55.5 ± 62.9 μg SRP/L, 320.5 ± 396.7 μg NH_4^+-N/L, 15.8 ± 2.5 μg NO_3^-+NO_2^- N/L) were high and variable, relative to previous observations (Francoeur et al. 2006), mainly because of large amounts of TP, SRP, and NH_4^+ present on day 73. Algae, bacteria, and fungi were all present in the litter-associated microbial communities, and fungal decomposers dominated the heterotrophic microbial biomass (>87%) (Table 1). In both experiments, algal communities were dominated by Chlorophyta, with Cyanophyta also abundant (Table 2). Heterokontophyta (mainly dia-

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Table 1. Mean (±1 SD) biomass of constituents of periphyton communities used in our study. In each experiment, algal and bacterial biomass $n = 4$ and fungal biomass $n = 10$.

Experiment	Algal biomass (mg chlorophyll *a*/g C)	Bacterial biomass (mg C/g C)	Fungal biomass (mg C/g C)
1	0.152 ± 0.07	2.23 ± 0.55	26.5 ± 11.0
2	0.197 ± 0.01	2.60 ± 0.20	18.5 ± 5.2

Table 2. Mean (±1 SD) relative abundance (% total cells) of algal divisions in microbial communities. $n = 4$ in each experiment.

Division	Experiment 1	Experiment 2
Chlorophyta	67 ± 13	66 ± 27
Cyanophyta	18 ± 13	32 ± 28
Heterokontophyta	14 ± 8	2 ± 4
Euglenophyta	<1 ± 1	<1 ± <1

toms) were common in experiment 1, but much less so in experiment 2. Euglenophyta were always very rare.

Microbial production and extracellular enzyme activity

Algal production (based on ^{14}C incorporation rates) in the presence of DCMU was not significantly different from 0 in either experiment (experiment 1: $n = 3$, $p = 0.524$, 95% confidence interval [CI] = −0.091 to 0.064 mg C g^{-1} C h^{-1}; experiment 2: $n = 3$, $p = 0.056$, 95% CI = −0.012 to 0.394 mg C g^{-1} C h^{-1}), although DCMU-based inhibition seemed slightly weaker in experiment 2. DCMU strongly inhibited algal photosynthesis; algal production in DCMU treatments always was significantly reduced relative to production in the DCMU-free treatment (p always <0.003; Table 3). In both experiments, the large DCMU treatment effect relative to small within-treatment variability resulted in great statistical power in the algal production experiments (Table 3).

DCMU exposure rapidly disrupted photosystem II electron transport activity. Even after only 5 min of exposure, photosystem II yield was significantly reduced ($p < 0.001$, ~75% reduction), with slightly larger reductions (~83%) observed after 10 ($p = 0.002$) to 15 ($p = 0.001$) min of exposure (Fig. 1).

In contrast to its strong inhibitory effect on photosynthesis, DCMU had no significant direct effects on bacterial production (p always >0.5; Table 3), fungal production (p always >0.34; Table 3), or extracellular enzyme activity (p always >0.08; Table 4). In all experiments, bacterial and fungal production were somewhat greater in the DCMU-amended than in the DCMU-free treatment, and enzyme activities were slightly elevated in the DCMU treatment relative to the DCMU-free treatment in 2 of the 4 experimental trials. The lack of any strong DCMU effect was reflected in the low statistical power of the bacterial and fungal production experiments and the enzyme activity experiments. Given the observed variability and negligible treatment effects, power analysis indicated that great numbers of replicates (bacterial production: $n = 254$–1860; fungal production: $n = 108$–112; enzyme activity: $n = 20$–62) would be required to achieve a 90% chance of detecting a statistically significant effect of DCMU (Tables 3, 4).

Discussion

Effects on algal photosynthesis

As expected, exposure of periphytic algal assemblages to 20 μM DCMU rapidly and effectively inhibited photosynthesis. Algal production rates in DCMU-amended wetland water were statistically indistinguishable from 0, whereas replicate communities in DCMU-free wetland water displayed robust photosynthesis. The time series PAM fluorometry measurements confirmed that inhibition occurred rapidly, with strong effects occurring within 5 min after exposure to DCMU.

Our results are consistent with those of other studies that report rapid inhibition of periphytic photosynthesis by DCMU exposure (Paerl et al. 1993, Neely and Wetzel 1995). Espeland and Wetzel (2001b) suggested that the slow (>1-d) onset of inhibition observed in their study was the result of the gradual addition of a DCMU solution into a large culture vessel filled with DCMU-free media and the presence of laminar flow conditions within the culture vessel. These conditions delayed the attainment of an effective DCMU concentration within the culture vessel, and they resulted in the presence of a thick diffusive boundary layer, which further delayed the exposure of periphytic microbes to DCMU (Espeland and Wetzel 2001b). Such conditions were avoided in our study because litter sections were immersed directly in 20 μM DCMU. Given the generally quick onset of inhibition (Paerl et al. 1993, Neely and Wetzel 1995, our study), a minimal preincubation exposure of 5 to 10 min seems adequate for ensuring that photosynthetic inhibition is complete before commencing with experimental measurements, as long as DCMU is added at a sufficient concentration (~20 μM) and transport of DCMU into periphyton is not unduly hindered by a thick diffusive boundary layer.

The biomass of the 3 microbial groups in each experiment was similar to those previously observed for submerged *T. angustifolia* litter in the Paint Creek

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TABLE 3. Mean (±1 SD) production of algae, bacteria, and fungi in periphyton in 3-(3,4-diclorophenyl)-1,1-dimethyl urea (DCMU) exposure experiments. Algal production was measured as mg C g^{-1} C h^{-1}, bacterial production as μg C g C^{-1} h^{-1}, and fungal production as mg C g C^{-1} d^{-1}. Percentage of change refers to the mean difference between treatments with (+) and without (–) DCMU. p values, power, and the replication (n) required for a power of 0.90 refer to 2-sample t-tests based on the experimental data, a 2-tailed null hypothesis, and $\alpha = 0.05$. PAR = photosynthetically active radiation.

Experiment	Periphyton component	Incubation PAR (μmol m^{-2} s^{-1})	n	Treatment +DCMU	Treatment –DCMU	% change	p	Power	n needed for power = 0.90
1	Algae	400	6	–0.014 ± 0.0311	0.373 ± 0.104	–104	0.003	0.993	6
	Bacteria	0	10	93.58 ± 16.04	82.11 ± 36.28	14	0.543	0.088	254
	Fungi	0	10	0.446 ± 0.161	0.351 ± 0.135	27	0.346	0.144	108
2	Algae	400	6	0.191 ± 0.082	0.667 ± 0.086	–71	0.002	0.998	6
	Bacteria	0	10	245.50 ± 52.49	229.96 ± 136.09	7	0.821	0.055	1860
	Fungi	0	10	0.609 ± 0.320	0.441 ± 0.210	38	0.361	0.139	112

wetland (Francoeur et al. 2006, Gillies et al. 2006). The relatively greater prevalence of cyanobacteria in communities in experiment 2 (32% vs 18% in experiment 1) might have contributed to the slightly (but nonsignificantly) greater ^{14}C incorporation observed in the presence of DCMU in experiment 2 than in experiment 1. Photosystem II-independent anoxygenic photosynthesis does occur in cyanobacteria (Garlick et al. 1977, Cohen et al. 1986) and can result in substantial ^{14}C incorporation in communities with abundant cyanobacteria and sulfide (e.g., Pinckney and Paerl 1997). However, anoxygenic photosynthesis is unlikely to have played any role in our experiments because of the requirement for sulfide as an electron donor. More important for our experiments, respiratory and photosynthetic electron transport chains are colocated on cyanobacterial thylakoid membranes and they share several components (Campbell et al. 1998). Thus, electrons from cyanobacterial respiration can fuel limited cyanobacterial photosystem I and Calvin cycle function even with complete inhibition of electron flow from photosystem II (Dominy and Williams 1987, Campbell et al. 1998). This phenomenon seemed to be relatively weak in our experiments because ^{14}C incorporation in the presence of DCMU was never statistically different from 0. Regardless, inhibition of electron flow from photosystem II will prevent O_2 production, even in communities dominated by cyanobacteria (e.g., Pinckney and Paerl 1997), and reliance on electrons from respiration to drive Calvin cycle function will prevent net organic C production because organic C must be mineralized to produce the electrons required for the fixation and incorporation of inorganic C.

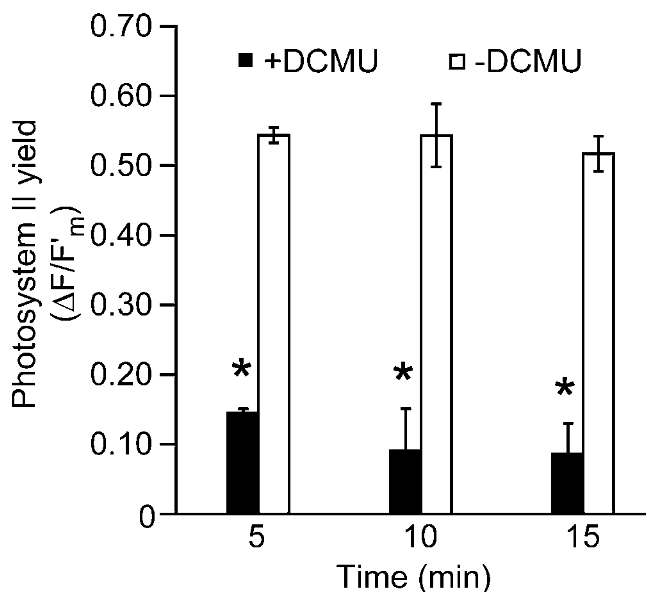

FIG. 1. Mean (±1 SD) light-adapted photosystem II yield (Gentry parameter = $\Delta F/F'_m$) of periphytic algae over 15 min of exposure to 20 μM 3-(3,4-diclorophenyl)-1,1-dimethyl urea (DCMU). Asterisks denote significant differences between treatments with (+) and without (–) DCMU within a sampling time.

The presence of a substantial cyanobacterial component also probably accounts for the small residual photosystem II yield signal observed in the presence of DCMU. In eukaryotes, fluorometric assay of photosystem II yield (i.e., the Genty parameter) is a good measure of photosystem II electron transport. However, it is only an approximate measure of cyanobacterial photosystem II electron transport because of the relatively large cyanobacterial photosystem I fluorescence signal and the wider variety of ways in which cyanobacteria can distribute electrons and photochemical excitation (Campbell et al. 1998).

Effects on bacteria, fungi, and enzyme activity

In contrast to its strong inhibition of photosynthetic activity, 20 μM DCMU had negligible direct effects on bacterial and fungal production. The small magnitude of any DCMU effect relative to the inherent variability

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Table 4. Mean (±1 SD; nmol g^{-1} C h^{-1}) extracellular β-glucosidase (BG), β-xylosidase (BX), phosphatase (Pase), and leucine-aminopeptidase (LAMP) activity in periphyton in 3-(3,4-diclorophenyl)-1,1-dimethyl urea (DCMU) exposure experiment 2. All measurements were made in the dark (photosynthetically active radiation [PAR] = 0 μmol m^{-2} s^{-1}). Percentage of change refers to the mean difference between treatments with (+) and without (–) DCMU. *p* values, power, and the replication (*n*) required for a power of 0.90 refer to 2-sample *t*-tests based on the experimental data, a 2-tailed null hypothesis, and α = 0.05.

Enzyme	*n*	Treatment		% change	*p*	Power	*n* needed for power = 0.90
		+DCMU	–DCMU				
BG	10	251.5 ± 26.7	296.8 ± 48.9	–15	0.106	0.360	24
BX	10	306.6 ± 154.8	231.2 ± 53.2	33	0.333	0.149	62
Pase	10	1309.1 ± 250.2	1699.4 ± 366.9	–22	0.085	0.409	22
LAMP	9	4892.6 ± 794.4	3911.0 ± 661.3	25	0.089	0.436	20

of bacterial and fungal production was highlighted in the power analysis. Assuming that our data accurately represent the DCMU effect size and within-treatment variability in bacterial and fungal production, 108 to 1860 replicates would be required to conduct experiments capable of detecting differences as small as those observed with a power of 0.9. The data do not support the hypothesis that DCMU inhibits bacterial or fungal production.

The presence of DCMU also had little direct effect on periphytic extracellular enzyme activity. In 2 of 4 experimental trials, activity was slightly depressed, whereas activity was slightly increased in the remaining 2 trials; no statistically significant effects were ever observed. The small magnitude of any DCMU effect relative to the inherent variability of periphytic extracellular enzyme activities was highlighted in the power analysis. Assuming that our data accurately represent the DCMU effect size and within-treatment variability in extracellular enzyme activity, 20 to 62 replicates would be required to conduct experiments capable of detecting differences as small as those observed with a power of 0.9.

Active algal photosynthesis can cause simultaneous increases in periphytic bacterial production (Murray et al. 1986, Neely and Wetzel 1995, Espeland et al. 2001) and periphytic extracellular enzyme activity (Espeland et al. 2001, Francoeur and Wetzel 2003, Francoeur et al. 2006, Rier et al. 2007). We conducted our heterotrophic production and enzyme activity assays in complete darkness to prevent the occurrence of any indirect influences of DCMU mediated by its inhibition of algal photosynthesis. Thus, our experiments measured the potential for DCMU to cause experimental artifacts during the analysis of heterotrophic microbial production and enzyme activity.

The lack of any strong, consistent direct DCMU effects on bacterial or fungal production and extracellular enzyme activity suggests that DCMU has minimal nontarget effects in periphyton communities. Our results are consistent with a previous study that reported a lack of any DCMU effect on heterotrophic bacterial production (Neely and Wetzel 1995). This apparent lack of nontarget effects, combined with good efficacy against algal photosynthesis and a relative ease of use, suggests that DCMU is a useful selective inhibitor for investigations of interactions among autotrophic and heterotrophic periphytic microbiota. Use of DCMU instead of light exclusion for photosynthesis regulation also could avoid any potential confounding bias of other light-sensitive processes.

DCMU affects only oxygenic photosynthetic activity because DCMU is a photosystem II inhibitor. Users should be aware that, under certain circumstances (e.g., sulfide-rich environments with abundant cyanobacteria or green and purple photosynthetic bacteria), photosystem II-independent anoxygenic photosynthesis is an important autotrophic pathway in periphyton (e.g., Pinckney and Paerl 1997). In such cases, DCMU-based protocols should be applied with care, and conclusions from such experiments must be restricted appropriately.

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