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Reductions in photosynthetic carbon uptake in epiphytic diatoms by water-soluble extracts of leaves of *Zostera marina*

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Abstract

A water-soluble fraction of green leaves of *Zostera marina* L. decreased carbon uptake rates in diatoms found as epiphytes at Roberts Bank (Lat 49°2′N; Long. 123°8′W) on the west coast of Canada. Extract concentrations of 11 to 1 μg dry leaf ml^{-1}, added immediately before ^{14}C-uptake was measured over a 3-h period, reduced uptake to 36 to 80% of controls, respectively. A lower concentration (0.6 μg dry leaf ml^{-1}) reduced uptake significantly in only one of four cultures. Addition of the whole extract 3 d before carbon uptake was measured or in one-third doses, 0, 1, and 2 d before, resulted in inhibition similar to that observed when the whole extract was added immediately before the ^{14}C.

Introduction

Harrison (1982) suggested that the chemical composition of eelgrass (*Zostera marina*) leaves might affect growth of the epiphytic flora. Microalgal epiphytes are efficient at trapping dissolved substances released from the leaves (Penhale and Thayer, 1980). Rates of loss of soluble organic compounds from seagrass leaves are low compared, for example, with macroalgae (Brylinsky, 1977; Penhale and Smith, 1977), but eelgrass leaves contain a range of water-soluble phenolic acids (Zapata and McMillan, 1979), some of which are potent inhibitors of the growth of marine bacteria (Harrison, 1982). The tests reported here show that epiphytic diatoms are also affected by soluble leaf components, exhibiting reductions in photosynthetic carbon uptake when exposed to extracts at concentrations in the parts per million range.

Materials and methods

Eelgrass collections and leaf extractions

Zostera marina L. plants were collected from the shallow subtidal flats at Roberts Bank (Lat. 49°2′N; Long. 123°8′W), British Columbia, Canada in August and October 1982 and August 1984. Plants were stored in the laboratory overnight in the dark in seawater at 5 °C. Shoots were rinsed carefully in freshwater to remove obvious epiphytes. About 100 to 200 g fresh weight of young, healthy leaves (usually the innermost four on the leaf bundle) were shredded in a Waring blender for 5 min at high speed with 300 ml methanol. The mixture was boiled for 15 min and then filtered through Whatman No. 1 paper. The leaf particles were re-extracted twice and all extracts were combined. The methanol was removed in a Buchler flash evaporator. The residue was dissolved in 50 to 100 ml distilled water and filtered through Celite diatomaceous earth to remove lipids. Extracts were frozen at −12 °C until used. Another portion of fresh leaves was blotted to remove surface water, weighed, dried to constant weight at 60 °C and reweighed for calculation of the ratio of dry to fresh weight.

Diatom cultures

A few unrinsed eelgrass leaves (2–4) were immersed in a flask containing 100 ml of a modified version of Provasoli's ES medium, the HESNW of Harrison *et al.* (1980). One milliliter of antibiotic solution containing penicillin G, streptomycin, and chloramphenicol (Hoshaw and Rosowski, 1973) was added to the flask to reduce bacterial growth. The flask was incubated on a rotary shaker (100 rpm) at 15 °C with a 10-h dark period and a 14-h light period under an irradiance of 64 μEin m^{-2} s^{-1} from cool white fluorescent tubes. After one week several flasks of fresh medium were inoculated from the original culture; subsequent transfers to new medium occurred every three weeks, but only cultures that remained free of obvious growth of bacteria, flagellates, and nematodes were kept. One week prior to each photosynthesis experiment, one culture was selected and divided among several flasks of HESNW medium for production of dense cultures. Three separate stock cultures were obtained from leaves collected

in August, September, and October 1982; each contained a mixture of naviculoid diatoms ranging from 20 to 80 μm in length and containing genera such as *Navicula, Gomphonema,* and *Diploneis* (Rao and Lewin, 1976). No obvious changes in the range of morphological types occurred during the culturing period. From plants collected in January 1985, four unialgal cultures were isolated using similar techniques as for the mixed cultures.

^{14}Carbon uptake experiments

A 2-ml inoculum of concentrated diatom culture (2 to 4 mg dry weight of diatoms) was added to 48 ml of HESNW medium. In one type of experiment, mixed cultures were used and leaf extract was added to duplicate or triplicate flasks at concentrations equivalent to 1.5 to 11.0 μg of dry leaf ml^{-1}. The extract and the diatoms were obtained from leaves collected at the same times ($\pm$ 3 wk). Two μCi of $NaH^{14}CO_3$ in 1 ml of water was added (keeping the flasks in the dark before and after addition), and all flasks were transferred to a rotary shaker for incubation as described above. After 3 h the lights were turned off and the contents of each flask were filtered through a different pre-weighed 0.45-μm Millipore filter under slight vacuum. Filters were dried at 60 °C for 18 h, reweighed, and placed into glass vials with 10 ml of Aquasol cocktail (New England Nuclear, Boston). Radioactivity was counted in a scintillation counter (Searle Analytic Inc. Isocap 1300, Des Plaines, IL) using an external standard channel ratio method with correction for background counts. Dark uptake was not measured nor were the data used to calculate true rates of photosynthesis; comparisons were made on the basis of dpm (disintegrations per minute) per mg of alga.

In the second type of experiment, unialgal cultures started in January were used. Leaf extract was added to seven or eight replicate flasks at concentrations equivalent to 0.6 to 2.0 μg of dry leaf ml^{-1}. The experiments otherwise paralleled those just described for mixed cultures. Since no difference was noted in the potency of extracts from leaves collected in different seasons, in this and subsequent experiments the extract used was from leaves collected the August prior to the initiation of the cultures.

In the third type of experiment, mixed cultures started in October were used and extract was added immediately before the $NaH^{14}CO_3$ (as described above); 3 d before; or in one-third doses, 0, 1, and 2 d before. When there was a delay between the additions of extract and of ^{14}C, the cultures were incubated in the normal manner. All treatments were run in triplicate.

Results

Addition of the leaf extract to a mixture of diatoms immediately before the ^{14}C was added resulted in a significant reduction ($p < 0.05$, Student's t-test) in carbon uptake over 3 h with concentrations as low as about 3 μg of dry leaf ml^{-1} (Table 1). Some, but not all, unialgal cultures were affected by even lower concentrations (Table 1). A strong negative correlation ($r = -0.9$) existed between mean carbon uptake (as percent of control) and concentration of extract.

There was no evidence of a recovery in ^{14}C-uptake when the extract was added at various times and dosages before the uptake was measured (Table 2). All treatments significantly reduced uptake rates ($p < 0.01$, one-way ANOVA), but no difference was found among treatments.

Table 1. *Zostera marina.* Inhibition of photosynthesis in cultures of epiphytic diatoms by leaf extracts measured over 3 h ($\bar{x} \pm$ SE). Extract was added immediately before the uptake of ^{14}C was measured. N = number of replicates. * = significant from control at $p = 0.05$ with Student's t-test

Month of leaf collection	Culture (mixed or isolate)	Leaf extract (μg dry leaf ml^{-1})	N	Carbon uptake (dpm mg alga^{-1})	
				With extract	Control
October	Mixed	11.0	3	6 284 ± 2 040 *	17 259 ± 857
October	Mixed	11.0	2	6 090 ± 874 *	15 318 ± 564
		2.8	2	9 383 ± 1 552 *	
August	Mixed	10.0	2	3 385 ± 612 *	7 006 ± 1 445
		3.3	2	4 862 ± 1 498 *	
		1.5	2	5 952 ± 1 288	
August	'A'	2.0	8	19 019 ± 807 *	30 051 ± 2 665
		1.0	8	22 828 ± 1 390 *	
	'A'	1.0	8	5 860 ± 667 *	7 928 ± 462
		0.6	8	7 094 ± 739	
	'B'	1.0	8	6 530 ± 301 *	8 725 ± 103
		0.6	8	8 138 ± 198 *	
	'C'	2.0	7	56 101 ± 4 243 *	86 020 ± 8 435
		1.0	7	68 758 ± 6 772	
	'D'	1.0	7	3 122 ± 355	4 000 ± 632
		0.6	7	3 722 ± 191	

Table 2. *Zostera marina.* Inhibition of photosynthesis in a culture of mixed epiphytic diatoms by August leaf extract added in one dose or three ($\bar{x} \pm$ SE; $N=3$). Uptake of ^{14}C was measured over 3 h on Day 4. ** = significant from control at $p=0.01$ with Student's t-test

Time of addition (d)	Extract added (μg dry leaf ml^{-1})	Carbon uptake (dpm mg alga^{-1})	
		With extract	Control
1	3.0	51 895 ± 8 529**	73 799 ± 4 927
2, 3, 4	1.0	53 696 ± 3 293**	
4	3.0	40 402 ± 5 907**	

Discussion

Rates of photosynthetic carbon uptake in three mixed cultures and in three of four unialgal cultures of epiphytic diatoms were strongly negatively correlated with the concentration of eelgrass leaf extract. The lowest effective concentration (equivalent to 0.6 ppm of leaf material) was less than one-tenth of that previously found to cause brief inhibition of growth of marine bacteria and the prasinophycean alga *Platymonas* sp. (Harrison, 1982). If, as seems likely from earlier work, the major phenolic constituents of the leaf were responsible, then those compounds were active at a concentration of about 10 ppb. Despite low rates of release of dissolved materials from eelgrass (Penhale and Smith, 1977), the fact that low doses of extract added over 3 d had the same effect as the same total amount added at the start or end of the 3-d period suggests that the epiflora are subject to long-lasting chemical influences from their substrate.

These experiments did not last long enough for obvious changes in the composition of the diatom assemblages to occur. As all the species cultured were isolated from eelgrass leaves, they must be tolerant to the chemical environment, but that environment varies. Specifically, the concentration of phenolic compounds declines as leaves age (Harrison, 1983, unpublished data). Diatoms were cultured from old leaves on which epiphytes are most abundant (Harrison, 1982), but extracts were made mainly from younger leaves. The variable response of the unialgal cultures to leaf extracts may indicate that the course of colonization of the leaves is influenced by the chemistry of the substrate.

It remains to be determined if other potential epiphytes never colonize eelgrass leaves because of an intolerance to the chemical environment. A re-examination of the data in Harrison (1982) suggests that bacterial isolates from seawater are more sensitive to eelgrass extracts than are bacterial isolates from the leaves. The process of colonization by individual algal species must be studied to determine if chemical inhibitors are an important mechanism whereby eelgrass reduces competition for light and nutrients by its epiphytes.

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