

# Activity of Naturally Occurring Hydrilla Growth Inhibitor: Initial Studies

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## ABSTRACT

Naturally occurring inhibitors of *Hydrilla verticillata* Royle have been isolated from sediment of Lake Starvation in Northwest Hillsborough County, Florida. Separation of the extracts by high performance liquid chromatography (HPLC) (methanol-water) yields two major components, one of which inhibits the rate of photosynthesis and accelerates the rate of respiration of hydrilla leaves in a Warburg apparatus. The implications for hydrilla management are considered.

*Key words:* hydrilla, natural products, inhibitor, photosynthesis, high performance liquid chromatography, respiration.

## INTRODUCTION

*Hydrilla Hydrilla verticillata* (Royle) is an exotic, rootable, submersed, perennial aquatic plant that was first identified in Florida waters in 1959-60 (8). Subsequently, it has caused serious hindrance to navigation and recreation in waterways of the southeastern United States (7) and it has spread into Texas and California (13). The plant has the capability of dominating the macrophytic community, owing to a mat-forming ability (7) and some highly adapted

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physiological properties (13, 14). The hydrilla control measures that have been used, including biocontrol with white amur or hybrid fish and chemical control with synthetic herbicides seem to have some disadvantages.

The possible existence of naturally occurring hydrilla growth inhibitors is attractive for potential use as a management tool. It is intriguing that certain lakes have been devoid of hydrilla or do not support its growth as well as might be anticipated. These lakes include White Trout Lake (10) and Lake Starvation (11) in Hillsborough County, Florida (near Tampa); Lake Seminole (Seminole County, Georgia) (2, 1); Lake Washington (King County, Washington) (2, 1); and Lake Kerr (Marion County, Florida) (9).

Our attention has focused upon Lake Starvation, where hydrilla has been present for about ten years, but it has not thrived. This lake has dark colored water and a considerable component of the lake sediment is the result of organic material from nearby stands of bald cypress *Taxodium distichum* (L.) Richard). Though aqueous extracts of the sediment inhibited the growth (5), aqueous extracts of branches and leaves of *T. distichum* did not (5). Thus, there is suggestive evidence that some natural products of bald cypress degradation serve as inhibitors of hydrilla growth and that these are produced in or on the sediment.

Current efforts are directed toward fractionating quantities of hydrilla inhibiting material through the use of high performance liquid chromatography (HPLC) and evaluating the mechanism of action of these fractions. The present communication describes the results of these efforts using HPLC fractionation and a Warburg apparatus for characterization of the effect of the fractions on hydrilla activity.

## EXPERIMENTAL

### Isolation of lake sediment extracts

Procedures used previously (5) were followed with slight modifications: 600 mL of distilled water was added to 300 g of wet sediment and autoclaved (110 C, 120 psi) for 20 min. The cool extract was filtered (Whatman #1), centrifuged (6,000 x g) for 10 minutes, and the centrifugate was filtered through an 8  $\mu$ m membrane filter in an all-glass filtering apparatus. Volume was reduced to 10 percent under reduced pressure using a rotary evaporator and keeping the bath temperature less than 35 C. The residue was passed over Bonding-Elut (C-18, Analytichem International) by centrifuging for 3 to 5 minutes on a clinical centrifuge.

### Fractionation of active components by HPLC

Material that passed through a Bond-Elut column was chromatographed with an Altex HPLC (Model 110) unit equipped with a solvent programmer attachment, a multi-wavelength detector and a preparative scale Zorbax column (21.1 x 35 mm). A linear gradient was used with a 20-minute run starting with 60% (v/v) methanol-water and finishing with 100% water.

### Effect of sediment extract on photosynthesis and respiration of hydrilla

Hoagland's solution (12) (5 mL supplemented with 400 mg of NaHCO<sub>3</sub> per litre) was pipetted into the outer annulus of Warburg flasks and leaves stripped from matched hydrilla stems were immersed in the solution. Central wells of the Warburg flasks were equipped with filter paper strips, saturated with NaOH solution to absorb CO<sub>2</sub>; the side arms contained the HPLC extracts, or water, in the case of control flasks. The atmosphere in the flasks was either N<sub>2</sub> for photosynthesis experiments or air for respiration studies. Flasks were immersed in a water bath at 30.5 C and shaken manually. Illumination (60  $\mu$ Es/m<sup>2</sup>/sec), as measured by a LiCor (Model Li 185A) radiometer/photometer, was provided by fluorescent lamps.

Manometer readings, measured as a function of time, were fitted to the best straight line by a least-square procedure. Slopes of the lines are a measure, in arbitrary units, of photosynthesis and respiration rates.

The initial rate of respiration/photosynthesis was measured in the flask as manometer readings for 30 to 60 minutes. Contents of the side arms were then added to the suspension of the hydrilla leaves and incubated in the dark for a time at 30.5 C. Measurements of photosynthesis/respiration rates were then repeated.

## RESULTS AND DISCUSSION

Following the scheme for extracting hydrilla inhibitor from Lake Starvation sediment, and using HPLC, two major fractions were collected (Figure 1), and both were tested for their activity on the rates of photosynthesis and respiration

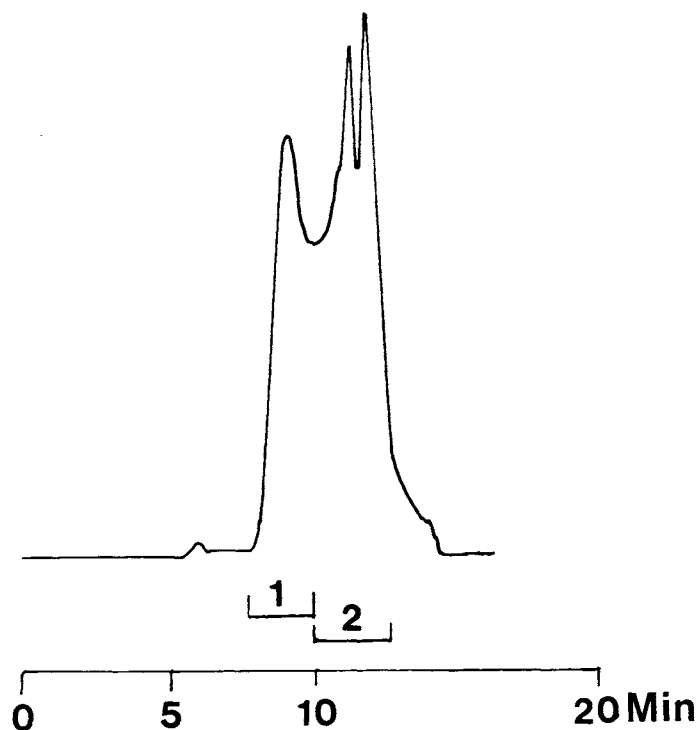


Figure 1. Typical HPLC chromatogram showing separation of hydrilla growth inhibitor into two fractions. Details for the isocratic mode using methanol-water are given in the text.

of hydrilla leaves. The results of the tests with the two fractions are summarized schematically (Figure 2) and in terms of relative rates (Table 1), i.e., (slope for test runs)/(slope for control run).

The first concerns were to verify that hydrilla leaves would maintain constant activity during the period of study, and that reproducible results could be obtained from one sample vessel to another of the Warburg apparatus. Both concerns were satisfied. First of all, the agreement between two runs was good. For example, consider the agreement between two samples, as demonstrated in Figure 2, (for photosynthesis), with slopes of  $0.090 \pm .005$  and  $0.089 \pm .004$  respectively for initial values. Secondly, the leaves maintained relatively constant activity for about 1.5 hours. For example, the initial slope for photosynthesis was  $0.090 \pm .005$ , and the value after 1.5 hours was  $0.081 \pm .003$ . On the other hand, after some additional 20 hours of standing, the value had decreased to  $0.057 \pm .003$  (see Figure 2). Thus, there is a limit to the length of time that studies using hydrilla leaves may usefully be pursued.

Fair precision was obtained, as reflected in the relative standard error, (Table 1), and was typically about 1 to 3% for a given run.

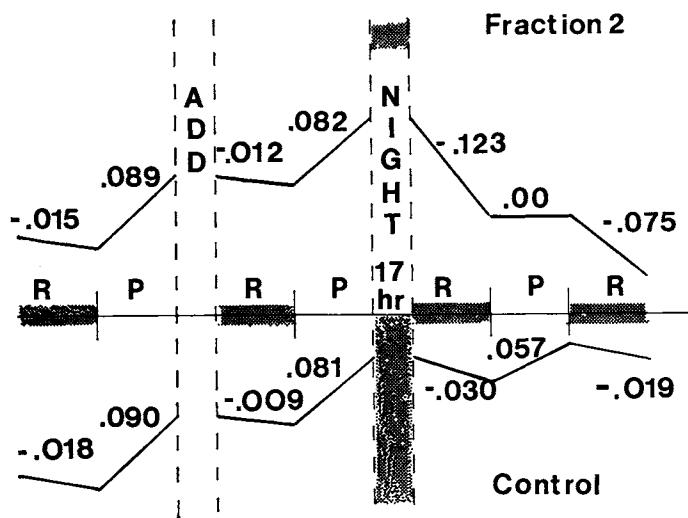


Figure 2. Schematic representation of the effect of hydrilla-growth-inhibiting material on relative rates of photosynthesis and respiration. The sequence shown indicates: (a) respiration, R, (b) photosynthesis, P, (c) addition of inhibitor, (d) respiration, (e) photosynthesis, (f) incubation overnight, 17 hours, (g) respiration, (h) photosynthesis and, (i) respiration. The effect of Fraction 2 is compared with a control sample.

TABLE 1. EFFECTS OF SEDIMENT EXTRACT (FRACTION 2) OF PHOTOSYNTHESIS AND RESPIRATION OF HYDRILLA LEAVES.

Concentration ppm	Incubation period, hr.	Relative Slopes <sup>a</sup>	
		Photosynthesis	Respiration
2	21	$0.72 \pm .04^b$	—
4	21	$0.66 \pm .04$	—
2	1	$0.75 \pm .06$	$2.35 \pm .42$
4	1	$0.72 \pm .18$	$3.92 \pm .89$
9	1	$1.18 \pm .099$	$4.89 \pm .02$
8	18	$0.016 \pm .014$	$3.95 \pm .005$
8	1	$1.01 \pm .045$	$4.03 \pm .04$

<sup>a</sup>slope test/slope control run  
<sup>b</sup>± standard error

In separate experiments, the effects of both fractions on photosynthesis and respiration were compared relative to a control sample (using distilled water). Fraction 1 had little effect on the photosynthetic rate and no effect on the rate of respiration of hydrilla leaves. Thus, any inhibiting effect on photosynthesis that was observed for fraction 1 was so small that the effect was probably ascribable to the contamination of fraction 1 by fraction 2.

The effect of adding fraction 2 to suspensions of hydrilla leaves was the reduction of the rate of photosynthesis, relative to control samples, and the acceleration of the relative rate of respiration. Two additional observations are pertinent. First, at times, the relative rate of photosynthesis was essentially unity. This may be the result of slow rate of diffusion of inhibitor to active sites. Upon standing, significant reductions in the relative rate of photosynthesis were observed (Table 1). Second, the effect of inhibitor depended upon the amount added, and the relative rate of photosynthesis decreased to 1% at the maximum concentration studied (8 ppm as organic carbon).

The results have several interesting implications. First, a convenient method of assaying activity is available. Second, the modes of inhibition of the growth inhibitor are now clearer, though the mechanism remains uncertain. Previous results have indicated the crude inhibitor is a singlet oxygen sensitizer (3). Whether this is the mechanism of action with fraction 2 is difficult to determine. Third, either mode of activity of fraction 2 (inhibition of photosynthesis or acceleration of respiration) is deleterious to the growth of hydrilla. Finally, the activity of fraction 2 is particularly promising in view of the fact that previous studies (6) indicated that the crude material did not adversely affect the growth of a common alga even to concentrations of 12 ppm (as organic carbon).

Obviously a number of problems remain unanswered. First and foremost, is the problem of separation. It is evident from a typical chromatogram (Figure 1) that, though a partial separation was effected, it was not a baseline separation. Fraction 2 appears to have at least two components on the basis of the chromatogram (Figure 1).

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