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Use of the EcoloGen[1] to Study Hydrilla Growth Inhibitors

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ABSTRACT

A commercially available apparatus (EcoloGen), has been used to culture hydrilla (*Hydrilla verticillata* Royle) and should be useful for studying the effect of phytopathic chemicals produced by organisms or extracted from sediments. The unit consists of four growth chambers mounted to a central chamber. Cultures of hydrilla or hydrilla and a second organism can be maintained in separate chambers separated from the central chamber by a membrane (test series) to permit diffusion of phytopathic products. Polyethylene film or glass plates can be used in place of the membrane to permit hydrilla and the other species to grow in pure culture (control series). The beneficial features include rapidity of screening, simulation of a natural environment, limiting organism-organism interaction to diffusion products with a second organism. Problems associated with the unit include mechanical problems, difficulties that can usually be solved without too much difficulty and sensitivity of some organisms to agitation, or to materials used in the EcoloGen. This latter group of problems may be extremely difficult to overcome.

Key words: allelopathy, natural products, diffusion, organism-organism interactions, multiple-diffusion unit.

INTRODUCTION

A number of lakes and waterways are classified as "hydrilla non-supportive". Approach to identifying these lakes and waterways has varied with the investigator. In some instances, the evidence is historical, being the observed disappearance of hydrilla or hydrilla-like plants (6) and the unwillingness to accept suggestions of loss of sexual potency or changes in nutrient levels as being the sole explanations. In other instances, the evidence is circumstantial (6,8,11) supported by evidence that extracts of lake sediment contained chemicals that inhibited the growth of hydrilla (4,6). In still other instances, lake sediments were used as substrates and tested for nutritional effects in very effective bioassays (1-3).

Based on earlier studies, the use of a multiple diffusion unit or EcoloGen seemed like a reasonable means of study-

[1]"ECOLOGEN" is a registered trademark of New Brunswick Scientific Company, Inc. Edison, N.J.

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ing the effect of phytopathic substances released by sediments or for studying the release of allelopathic compounds. The EcoloGen was invented by Tannenbaum and Kornfeld (13); and it is a commercially available unit designed for investigating mixed-culture interaction. It consists of a cubic clear plastic central diffusion reservoir to which are mounted four Pyrex growth chambers (Fig. 1a). Each chamber consists of a 300-ml cylindrical jar with a threaded neck, which is used to attach the jar to the cubic mixing chamber. Each culture chamber can be separated from the mixing chamber by either a membrane filter, which permits diffusion of chemicals, or a glass plate that isolates the culture chamber (Fig. 1b).

This study examines the use, advantages and limitations of the EcoloGen as applied to the study of hydrilla and its interactions with a naturally occurring inhibitor (5,7).

MATERIALS AND METHODS

The EcoloGen was purchased from New Brunswick Scientific Co., Inc., Edison, New Jersey, as a four chamber unit.

Procedures used previously (4,5) were followed using sediment from Lake Starvation, Hillsborough County, Florida. A crude extract was used and contained 637 ppm organic carbon, as measured by a Beckman Model 915 total carbon analyzer.

The growth medium used was 10% Hoagland's solution (12) modified by the addition of 5 ppm potassium bicarbonate. Hydrilla was obtained from the University Square pond near 30th Street in north Tampa. The plants were stored in tap water then stored overnight in deionized water, shaken dry, and weighed. Two shoots (1.2-2.5 g total fresh wt.) were used in each of the four growth chambers. The chambers were separated from the central reservoir by stainless steel screens (standard) instead of membranes. The reservoir and chambers were filled with the growth medium and the entire assembly placed on a rotary laboratory shaker (Model G-2, New Brunswick Scientific), and the shaking was adjusted to 75 rpm. Lighting was provided by an overhead light and four small cool-watt fluorescent lamps placed so that the light at each jar was 70 $\mu E/m^2/sec$ on a 12-hr light-dark cycle.

The experiment was run for 14 days. At the end of the runs, the hydrilla shoots were carefully removed from the chambers, combined and weighed. The percent change in fresh weight was determined.

To study the effect of the inhibitor, two chambers to control samples were sealed off from the central diffusion chamber by plastic disks. The other two chambers were connected as previously described, and 3 ml of the inhibitor extract (providing 1.6 ppm C_{org}) was added to the medium in the central chamber. The experiment was run for one week, with shaking and lighting as before. The results are shown in Table 1, study III.

In subsequent experiments, the effect of inhibition was tested using 20 g of sediment placed in the central chamber with 500 ml of medium. The control chambers were sealed off as before, and the test chambers were connected with 0.45 μ Nuclepore membrane filters. After two weeks, the fresh weight of the hydrilla samples was measured.

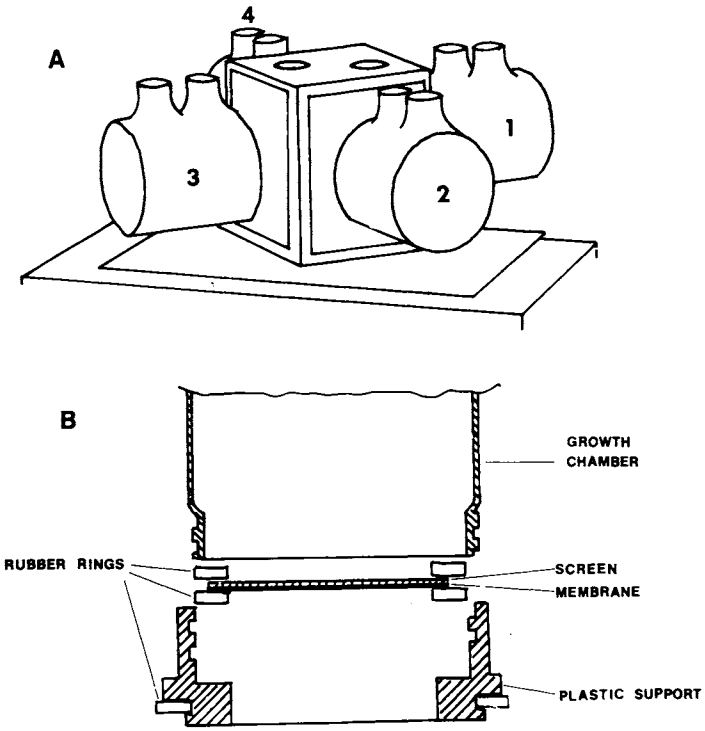

Figure 1. (A) Schematic representation of the multiple diffusion chamber (EcoloGen). The base is 13 cm × 13 cm, each chamber holds 300 ml, and the diameter is 7 cm with upper ports (each 2 cm in diameter). Chambers are mounted to the central chamber by screws. The volume of the plastic central mixing chamber is 500 ml. The entire unit is bolted to a NBS model C-2 laboratory rotator (28 × 32 cm). (B) Details of the mounting of individual culture flasks. The stainless steel screen is perforated with holes that are about 0.8 mm in diameter.

RESULTS AND DISCUSSION

Certain mechanical-biochemical problems become evident in using an EcoloGen, and these need to be addressed early in a study. Uniform lighting is essential of course with a macrophyte; otherwise, shading can occur even with the unit mounted on a gyro-rotary shaker. In the instance of hydrilla, the problem of shading was controlled by using short apical portions as well as uniform overhead and horizontally arranged lighting. A more serious matter in some cases is how fastidious the organism(s) being studied is and its incompatibility with materials in the unit or the conditions (shaking). Such was the problem with *Ptychodiscus brevis* (10), which died and may have been sensitive to gentle shaking, or may have been incompatible with materials in the gaskets (using different kinds of rubber did not prove to be effective).

The problem of adequate number of replications is especially significant with an EcoloGen, which has a limited number of replications available. Initial checks indicated a fair degree of uniformity in the growth of hydrilla in Hoagland's solution. The percentage increase in fresh weight was consistent (mean for three studies, 42 ± 7%; Table 1). In addition, there was no statistically significant difference ($P = > 0.05$) in hydrilla growth between any two studies using the EcoloGen. Fair relative standard errors were noted, the best being 10%.

The effect of naturally occurring hydrilla inhibitor (5,7) on percent hydrilla fresh weight increase was studied

TABLE 1. SUMMARY OF STUDIES WITH CHANGE IN FRESH WEIGHT CHANGE OF HYDRILLA MAINTAINED IN AN EOLOGEN

Study	Study Description	% Change in fresh weight[a]	P[b]
I	4-control replicates	43 ± 7	
II	4-control replicates	36 ± 5	
III	2-control replicates	49 ± 8	0.025>P>0.01
	2-test replicates (exposed to inhibitor)	13 ± 1	
IV, V[c]	2-control replicates	34 ± 3	0.025>P>0.01
	2-test replicates (exposed to suspended sediment in central chamber)	14 ± 6	

[a]Mean ± SE.
[b]Comparison of test and control.
[c]Pooled data from two experiments.

briefly (Table 1, study III). The difference in growth between the control and test samples was statistically significant ($P = < 0.025$). For control samples, the mean and standard error in fresh weight change was 48 ± 8%, and the corresponding values for hydrilla, which was exposed to inhibitor in the test jars, were 13.5 ± 0.5%.

In addition, Lake Starvation sediment was suspended in Hoagland's in the central chamber, and the results indicate that the increase in fresh weight of hydrilla in the test chambers was significantly less than in the control chambers (Table 1, studies IV and V). This result is consistent with the previous observations (9) that hydrilla growth inhibitors could be isolated by extraction of Lake Starvation sediment at room temperature. With EcoloGen experiments, we have demonstrated a useful technique for evaluating the effect of aqueous extracts of sediments (or potentially, allelopathy) on hydrilla and other suitable plants.

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