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Effect of a Naturally Occurring Growth Inhibitor on the Ultrastructure of Hydrilla

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INTRODUCTION

Previous papers have described our efforts to characterize a naturally occurring hydrilla inhibitor. In 1980, it was noted that aqueous extracts of sediment from Lake Starvation, located in Hillsborough County, Florida, inhibited the growth of hydrilla (1). The implications of this information were summarized (2) in a review of allelopathy and the natural production of phytopathic substances. Subsequently, it was demonstrated that it was possible to characterize the presence of inhibitor in other water samples through the use of high performance liquid chromatography (3). The mode of action of the inhibitor was investigated, and evidence suggested that the active material suppressed photosynthesis and increased the rate of respiration (4). The inhibitor can serve as a sensitizer for production of singlet oxygen, and one mode of action may be through photodynamic action (5).

The present study is concerned with the effect of the inhibitor on the ultrastructure of hydrilla as revealed by electron microscopy. Previous studies of the ultrastructure of hydrilla are available for guidance (6, 7). None of these studies have focused upon the changes that occur as a result of the presence of growth inhibitors, but the effect of herbicide treatment on ultrastructure has been well studied in other plants (8).

MATERIALS AND METHODS

Procedures used previously (1, 4) were followed using sediment from Lake Starvation, Hillsborough County, Florida. A crude extract was used that contained 637 ppm organic carbon, as measured by a Beckman Model 915 total carbon analyzer.

The inhibitory activity of the extract utilized in this experiment was determined by measuring the effect of the inhibitor on the respiration rate of hydrilla leaves, using an oxygen monitoring system (Yellow Springs Instruments, Model 53). Ten matched hydrilla leaves and 5-ml of 10% Hoagland's solution (9) were placed in control and test chambers. The bath assembly was maintained in the dark at 30°C. Readings were taken for 60 min, the inhibitor (0.2-ml) added to the test chamber, and readings continued for another 90 minutes. The leaves were left in inhibitor overnight (20 hrs, 20 min) and readings were taken for another 60 min. The leaves incubated in the inhibitor exhibited a significantly higher ($P < 0.01$) rate of respiration confirming the inhibitory activity of the sediment extract.

Hydrilla was treated with inhibitor and prepared for microscopy. Two 500-ml Erlenmeyer flasks were filled with Hoagland's solution. The experimental flask had inhibitor added to a final concentration of 4%. To both flasks were added four apical ends of hydrilla. The leaves of one apical end were prepared immediately for electron microscopy (Day 0, control). The flasks were stoppered, inverted, and illuminated (105 $\mu Es/m^2/sec$, as measured at the flask; 12 hrs). On each of Days 1 through 4, an apical tip was removed from each flask and prepared for electron microscopy.

The procedure for electron microscopy followed Pendland (6). Leaves were cut with a razor blade in sections <1-mm, fixed according to Karnovsky (10), and embedded in Spurr's low viscosity medium (11). Blocks were sectioned on a Sorvall "Porter-Blum" ultramicrotome, poststained with uranyl acetate (12) and observed using a Phillips 200 Electron Microscope.

RESULTS AND DISCUSSION

Beginning as early as Day 1, chloroplasts from leaves of hydrilla incubated in inhibitor exhibited an accumulation of starch not seen in chloroplasts in control plants. The same results were observed in the control and inhibited plants after three days of incubation. After four days of incubation, the chloroplasts of inhibitor-treated leaves (Fig. 1b) were markedly distorted in their appearance, owing to starch accumulation. This effect was not found in the leaves from control plants (Fig. 1a).

The amount of starch normally stored in leaves may be controlled by a partition of photosynthate between starch and sucrose. The mechanisms involved in this control are not yet understood (13).

It appears that the hydrilla growth inhibitor may be involved in either the disruption of the partition mechanism causing excess starch accumulation or in preventing the normal degradation of starch to soluble products. This study should be useful in guiding future research in the management of hydrilla by naturally occurring inhibitors.

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Figure 1a (left). Hydrilla leaf chloroplasts after four days in Hoagland's solution. X 12,833. lb (right). Hydrilla leaf chloroplasts after four days in Hoagland's solution with inhibitor. X 22,200.

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