Effect of Atrazine on Algal Contamination and Sugarcane Shoots During Photoautotrophic Micropropagation

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Abstract. *A laboratory procedure was developed for obtaining and maintaining photoautotrophic cultures of sugarcane shoots in vitro for three generations. Algae contamination in vessels was a problem for photoautotrophic growth under septic conditions. Atrazine, a herbicide, was effective in controlling algae at a concentration of 10 ppm, but the growth of shoots in herbicide–treated sugarless Murashige and Skoog (MS) medium decreased to about half of the growth in untreated medium. As a result, atrazine was not considered to be a practical solution for algae control. Additional work is needed to optimize photoautotrophic micropropagation of sugarcane for both septic and aseptic conditions. Photoautotrophic micropropagation of sugarcane may be economical only if culture can be perfected under septic conditions.*

Keywords. *Algae, Atrazine, Contamination, Hydroponic, Tissue culture.*

This article is the third in a series of research reports on the photoautotrophic micropropagation of sugarcane shoots. First, the effect of growth factors such as light, carbon dioxide (CO_2) levels in the growth chamber, and hormone composition of the growth medium on transformation to photoautotrophy of sugarcane shoots in micropropagation was studied (Erturk and Walker, 2000a). Then, improving the conditions for photoautotrophic micropropagation of sugarcane plantlets, during and after transformation to photoautotrophy, was analyzed (Erturk and Walker, 2000b).

Algae growth was observed in the experiments that were performed in the septic vessels with hydroponic solutions placed in the septic environment of the growth chamber (Erturk, 1998). The environment was naturally susceptible to algae growth because of the aqueous medium, high light levels, and high nutrient concentrations. Very little algae growth was observed in the MS (Murashige and Skoog, 1962) medium, and the type of algae in the MS medium was different in terms of color than that seen in a Hydro–sol medium. The reason was probably because the pH and the nutrient concentration of the two media were different from each other. A herbicide, atrazine, was tested as a solution to the problem.

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Literature Review

Plumley and Davis (1980) studied the effect of atrazine on algae in culture, in microecosystems, and in the field. A 10^{-5} M atrazine concentration reduced the rate of photosynthesis, chlorophyll content, and cell numbers in unialgal cultures isolated from a salt marsh habitat. Results with lower atrazine concentrations indicated an ability to maintain chlorophyll production and cell division with reduced photosynthesis. The effects of atrazine in unialgal cultures were also evident in microecosystems and in the field at the same concentration, but atrazine effects were more severe in microecosystems or cultures than in the field.

Christopher and Bird (1992) reported in their work with the tissue cultures of aquatic plant *Myriophyllum spicatum* that atrazine significantly decreased the length of the new leaves and branches with concentrations greater than 20 ppm, resulting in production of abnormal (curled leaves) or shortened leaf length. *M. spicatum* development in tissue culture occurred by growth at the nodes, initially by production of axillary buds that contain a shoot meristem or roots. At 100 ppm atrazine, the plants frequently appeared yellow or chlorotic.

The main objective of the overall study was to demonstrate photoautotrophic micropropagation of sugarcane under septic conditions. A septic, liquid propagation system using inorganic nutrients was proposed. The presence of any algal contamination would out–compete sugarcane for inorganic nutrients because algae would grow rapidly, compared with sugarcane. The specific objective of this article was to present an analysis of the algal contamination problem. While the algae problem was being addressed, other phases of the study continued in parallel using aseptic culture.

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Materials and Methods

Sugarcane Stock Culture

The sugarcane stock culture was as described by Erturk and Walker (2000b).

Growth Chamber

The growth chamber and the culture room were as described in Erturk and Walker (2000b).

Media

Stock and gelled MS (Murashige and Skoog, 1962) media were as described in Erturk and Walker (2000b). The sugarless MS medium had the same ingredients as the gelled MS medium except that the sugar and Phytagel™ were removed from the medium. The sugarless MS medium was used in the liquid (without Phytagel™) form except where noted.

The hydroponic medium used for the photoautotrophic growth studies was a commercially used, full–strength Hydro–sol (Grace Sierra Horticultural Products Company, Allentown, Pa.), which consisted of basic inorganic nutrients used for growing most plants in hydroponics. The hydroponics solution was prepared by dissolving 1.2 g of Hydro–sol and 0.9 g of calcium nitrate in 1 L of water.

Experiments

Atrazine concentrations (0, 50, 100, 150 and 200 ppb) in Hydro–sol solutions were used for the preliminary experiment with no sugarcane shoots. There were two vessels for each treatment, having 200 mL of Hydro–sol solution each. The vessels were inoculated with 1 mL of liquid from a wild algae culture. The algae culture was a naturally occurring population obtained from open containers of Hydro–sol solution kept in the growth chamber under relatively high levels of artificial lighting.

Based on visual observation, none of the above treatments were able to inhibit the growth of algae after one week. Higher concentrations of atrazine were then tried. Concentrations of atrazine used were 0, 0.2, 1, 10, and 100 ppm in Hydro–sol solution. There were ten replicates for each treatment. After one week, atrazine at 100 ppm apparently killed algae originally inoculated in the vessels, and the 10 ppm treatment was found to inhibit the growth of algae.

These preliminary experiments were used primarily to determine the range of atrazine concentrations that inhibit algae growth. The main experiment was then conducted to test how atrazine affects the growth of sugarcane shoots. Experiment treatments were selected to be 0, 10, 20, and 50 ppm atrazine. In other experiments, MS medium was found to be the most suitable medium for photoautotrophic growth in a concurrent experiment (Erturk and Walker, 2000b); therefore, the sugarless liquid MS medium was used for the current experiment. Four shoot clumps of sugarcane were subcultured to the vessels with sugarless medium + atrazine. There were 10 replicates for each treatment. Measurements of fresh weight of shoots determined if sugarcane growth was inhibited in any of the above treatments after two weeks.

Table 1. Results (wet weight in grams) of sugarcane growth with and without atrazine, including standard deviations in parentheses.

Treatment	Average Initial Weight	Average Final Weight	Average % Increase in Weight
0 ppm atrazine (control)	0.50 (0.10)	1.93 (0.33)	286 (95)
10 ppm atrazine	0.48 (0.08)	0.81 (0.14)	69 (27)
20 ppm atrazine	0.47 (0.08)	All dead	—
50 ppm atrazine	0.52 (0.09)	All dead	—

Results and Discussion

The results from the atrazine test are shown in table 1. The 20 and 50 ppm atrazine treatments killed the sugarcane shoot cultures grown in MS liquid medium. The 10 ppm atrazine treatment did not kill the plants but decreased the growth of the sugarcane shoots to less than half, compared to the no–atrazine medium. Fading in color of the shoots was observed in the atrazine–treated cultures.

Statistical treatment of data was performed using ANOVA methods. The differences between the initial and final weight data were evaluated to determine if the differences were statistically significant. The effect of atrazine concentration on the growth of sugarcane shoots was found to be statistically significant.

Because atrazine interferes with photosynthetic electron transport, the fading in color of the shoots grown in medium with higher concentrations of atrazine was not surprising. The shoots died eventually since they were not able to photosynthesize in the absence of exogenous carbohydrates. The above results seem to be consistent with the results of Christopher and Bird (1992), i.e., concentrations $\geq$20 ppm caused the death of shoots, as observed first by fading in color and eventually browning of the shoots.

Conclusion

Although atrazine was found to be effective as a control agent for algae growth, it was not found to be a practical solution overall because of the decreased shoot growth. Atrazine at 10 ppm was found to be marginally effective in controlling algae, but it reduced sugarcane shoot growth by more than half. Algicides, other than atrazine, would be worth trying as a means of avoiding algae growth in the aqueous medium. Cupric sulfate can be considered as an alternative to kill algae by increasing the copper concentration in the growth medium. Due to the time limitations of this work, we were not able to study the effects of cupric sulfate, but we suggest that it be tried in the future.

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