

THE INFLUENCE OF THE ISOPROTURON HERBICIDE ON GROWTH, GASEOUS EXCHANGES AND ASSIMILATORY PIGMENT CONTENTS IN *BOTRYOCOCCUS BRAUNII* Kuetz.

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ABSTRACT

The reactions of *Botryococcus braunii* Kuetz. cells treated with an urea compound, isoproturon, were investigated. The effects on cells number, growth, assimilatory pigment content and gas exchanges were recorded. Isoproturon was added in Zehnder-Gorham nutritive medium of algal suspension in increasing concentrations from 0.04 μM to 3 μM . For the concentration of 0.04 μM isoproturon, in the first period of the culture cycle, a relative stimulating effect on growth, assimilatory pigment content and photosynthesis was noticed. Respiration was affected at all used concentrations. For the first 2 days, the isoproturon doses which decrease with 50% the growth of the cells number of *Botryococcus* were about of 5.61 μM and for the 21 days from the culture cycle the concentration which causes this effect diminishes at 0.82 μM . When the pigment content was reported to number of cells per ml no direct influence of isoproturon was noticed, but only an adaptive reaction to self-shading.

Key words: assimilatory pigments, *Botryococcus braunii* Kuetz., growth, isoproturon herbicide, photosynthesis, respiration.

INTRODUCTION

Herbicides play an important role in agriculture and demand for them is increasing. These phytotoxic compounds block metabolic pathways essential for plant growth (Mori et al., 1995).

Phytotoxic symptoms occur during growth, and these effects can be advantageously followed and quantified with algae or cell cultures. Thus, the unicellular algae represent an useful experimental material for studying the herbicide impact on plant physiology (Böger and Sandmann, 1990). The results obtained by studying the herbicide action on the unicellular algae can be used for understanding

the herbicide effects on higher plants (Schäfer et al., 1994).

This article presents the effects of an ureic compound, isoproturon (N-4-isopropylphenyl) N', N'-dimethylurea) on growth, gas exchanges and assimilatory pigment content in *Botryococcus braunii*.

MATERIALS AND METHODS

Botryococcus braunii algae were cultivated on Zehnder-Gorham nutritive medium (Vladeanu et al., 1988). The experiments were carried out in a chamber with artificial illumination of 8000 lux. The ambient temperature varied between 24-25°C. To avoid mutual shading, the algal suspensions, in cylindrical glass recipients of 1,000 ml, were bubbled with a steady stream of air produced by vibrator devices. The culture medium was inoculated with an amount of algal biomass producing a 100,000 cells/ml suspension in all experiment variants. The results represent average values of three replications for each variant.

The isoproturon herbicide was introduced in the culture medium before inoculation and the concentrations of the tested herbicide solutions varied from 0.04 μM to 3 μM .

The commercial product Izoguard was used as a source of isoproturon. The Izoguard herbicide has 50% active substance (Arape, 1987).

The growth of algae was observed at each two days within the culture cycle (established at 21 days) with a "Cecil 1020" spectrophotometer at $\lambda = 676 \text{ nm}$. A strong quadratic relationship ($R^2=0.993$) was found between extinction

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and the number of cells counted with a Thoma haemocytometric mount (Figure 1).

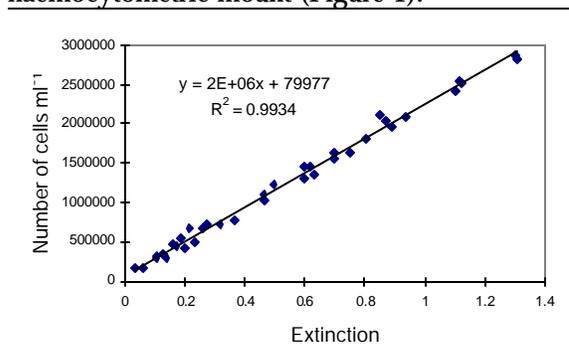


Figure 1. The relationship between extinction and the cell number/ml

The areas (A) encompassed under the graphs for the number of cells/ml (X) from each concentration (c) at different days (t) were calculated with the following formula:

$$A_{(c,t_n)} = \sum_{i=0}^n (t_{i+1} - t_i) \times \frac{[x_{(c,t_i)} + x_{(c,t_{i+1})}]}{2}$$

for $t = 2, 14$ and 21 days.

For each replicate, several simply non-linear regressions were performed between the reductions (% from control) of these areas (x values) and the doses of herbicide as y values. The equation: $y = a + bx^3$ was preferred because the confidence intervals were narrower than those obtained by other types of equations which produced better values for R^2 . The equations for each replicate were used to interpolate the LD 0, 25, 50, 75, 100% for these replications in order to calculate the standard deviations. The regressions for all the replicates from a given day were used to calculate a common R^2 . The interpolated values with these equations were similar with those obtained from replicates. This mathematical treatment integrates the influence of each herbicide concentration over the whole duration of experiment.

The photosynthesis rate was investigated with Warburg method using the treated algal suspension and the Warburg buffer solution number 9 (15 ml Na_2CO_3 0.1 M + 85 ml NaHCO_3 0.1M) (Boldor et al., 1983).

The respiration rate was established with Warburg method using the treated algal suspension and a solution of KOH 30% to capture the CO_2 resulted in the respiration process (Boldor et al., 1983).

The absorbance of the solutions extracted with 100% acetone was measured at 661.6, 644.8 and 470 nm and the calculation of the content in a, b chlorophylls and carotenoids was performed according to Lichtenthaler (1987); the values were expressed in mg/g dry matter (DM).

RESULTS AND DISCUSSIONS

The influence of isotroturon on growth in each treatment was observed from the second day after herbicide application. For the concentration of 0.04 μM isotroturon a relative stimulating effect was noticed, but the growth rate did not differ significantly from that of the control excepting the 16th day of the culture cycle. In the last two days of the experiment the cell number decreased under the control level for the concentration of 0.04 μM isotroturon.

The growth depression was proportional to the applied dose and, excepting the first 4 days of the experiment, there were clear cut differences between treatments especially between 0.2 μM and 0.4 μM concentrations and between 0.9 μM and 1.2 μM isotroturon. The treatment with 3 μM isotroturon induced a small increase in the cell number in the first week of the experiment. After that, it remained at an almost constant level (Figure 2).

The procedure described in "Material and methods" was used to fit the a and b empirical parameters for equation $y = a + b * x^3$, where the x values are the differences from control for the areas under the growth curves (%) and y is the herbicide dose which is expected to produce this effect.

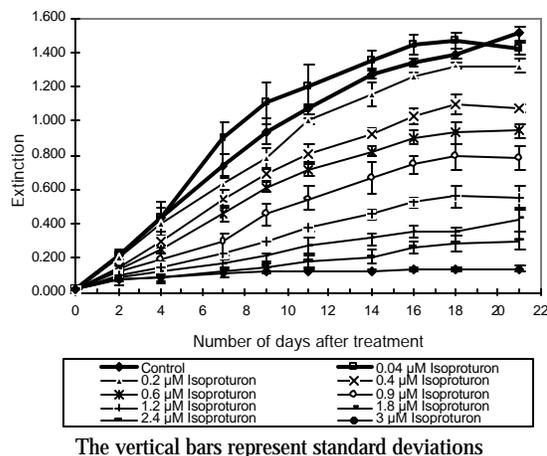


Figure 2. The influence of isoproturon on extinction of *Botryococcus braunii* culture

The lethal concentrations of isoproturon for 2, 7, 14 and 21 days calculated with the described method are shown in table 1.

A very good resistance of *Botryococcus braunii* at isoproturon was observed especially in the first two days of the experiment. It was demonstrated the existence of a resistance mechanism to this herbicide relied on the hydroxylation of the third carbon from the rest of the isopropyl from the isoproturon molecule with the aid of the P 450 cytochrome (Reichert et al., 1982, quotation by Durst, 1991).

The photosynthesis rate for the 0.04 μM treatment was higher than that obtained for the control for the first two weeks, but without significant differences. We can't consider this observation like a stimulation of the photosynthesis. In the last week of the experiment the 0.04 μM treatment affected the photosynthesis intensity, especially in the last day of the culture cycle. There were clear cut differences between treatments in the last part of the culture cycle and the 1.8 μM treatment detached evidently from the other concentrations (Figure 3).

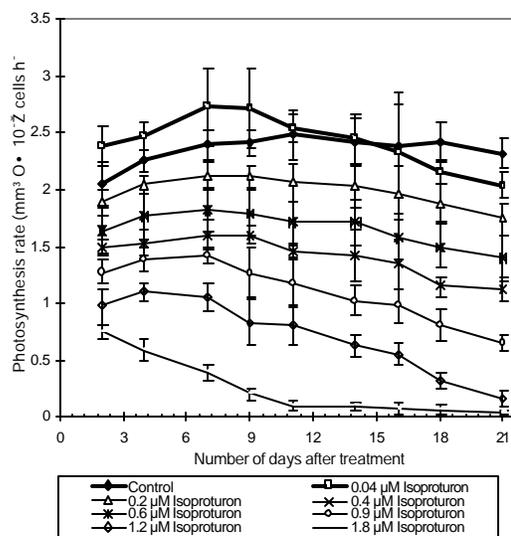


Figure 3. The influence of isoproturon on photosynthesis rate of *Botryococcus braunii*

The respiration depression was proportional to the applied dose. For the 0.4 μM treatment the respiration rate was not significantly different from 0.6 μM and 0.9 μM doses and between 1.2 μM and 1.8 μM concentrations there were no significant differences only in the last 12 days of the culture cycle (Figure 4).

As regards the influence of isoproturon on the assimilatory pigment content the observations showed that the 0.04 μM concentration presented higher values than control in the 7th and 10th days of the culture cycle. In the 14th day of the experiment these values decreased under the control values, but without significant differences and in the 20th day the assimilatory pigment content was evidently under the control values. For the other concentrations the assimilatory pigment content showed a decrease from control for the entire experiment and this depression was proportional to the applied dose (Figure 5 a, b, c).

Table 1. Isoproturon growth inhibitory concentrations (μM) for *Botryococcus braunii*

Days	2		7		14		21	
	Averages	SD*	Averages	SD*	Averages	SD*	Averages	SD*
0	0.127	0.111	0.137	0.039	0.192	0.021	0.272	0.022
25	0.814	0.013	0.238	0.027	0.267	0.017	0.341	0.020
50	5.618	0.676	0.950	0.068	0.794	0.034	0.823	0.031
75	18.659	2.547	2.881	0.317	2.222	0.146	2.131	0.109
100	44.053	6.190	6.641	0.805	5.003	0.368	4.678	0.270

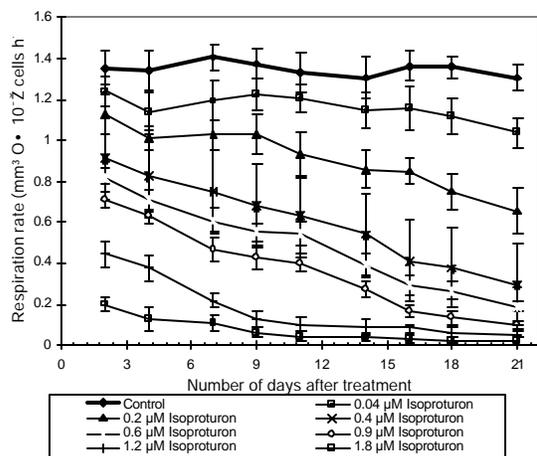


Figure 4. The influence of isoproturon on respiration rate of *Botryococcus braunii*

The correlation between the cells number and the assimilatory pigment content suggests that the influence of the isoproturon on the assimilatory pigment content is indirect and the recorded variations may be explained only by an adaptive reaction to self-shading.

The results show that the isoproturon herbicide induced a quick and sharp limitation of the physiological activity related with algal cell growth in liquid suspension.

CONCLUSIONS

Generally, for low concentrations in the first growth period a relative stimulating effect was noticed, but soon, an obvious depression appeared in the evolution of the investigated phenomena. Thus, isoproturon is a toxic herbicide (LD 50% after 2 days was 5.618 μM and LD 50% after 21 days dropped at 0.823 μM isoproturon).

In regard of the gas exchanges, an inhibitory influence of the isoproturon herbicide on the photosynthesis and respiration rate was observed.

When the pigment content was reported to the number of cells per ml no direct influence of isoproturon was noticed, but only an adaptive reaction to self-shading.

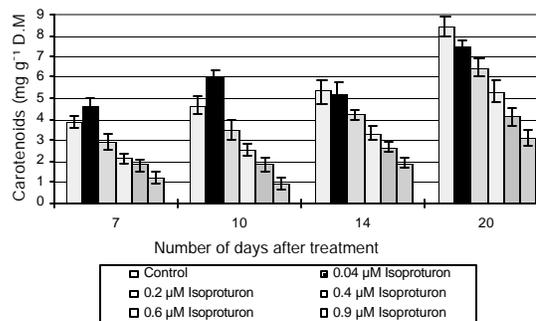
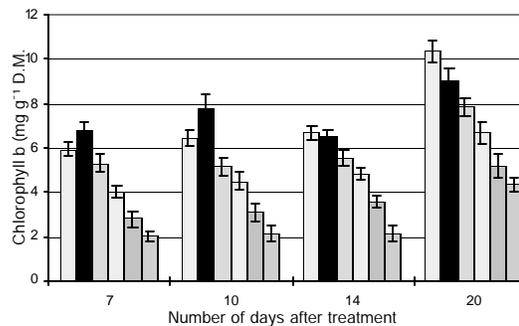
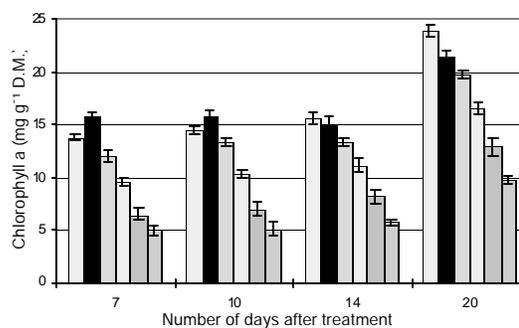


Figure 5. The influence of isoproturon on a and b chlorophyll and carotenoid pigment content in *Botryococcus braunii*

Acknowledgements

This study was supported by the Romanian Ministry of National Education through a CNCSIS grant (37116/2000).

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