632.954 Simetone + DCMU: 581.132

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THE EFFECT OF THE HERBICIDES SIMETONE AND DCMU ON PHOTOSYNTHESIS

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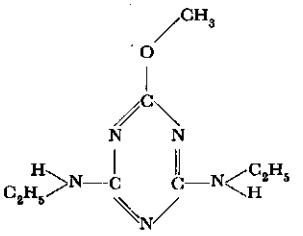
INTRODUCTION

Work on the effect of herbicides on photosynthesis was started in this laboratory in 1959 by the second author, who measured the inhibition of photosynthesis in suspensions of unicellular algae by Chloro-IPC, Monuron and Simazin, and estimated the effect of these compounds at different light intensities (VAN STEEKELENBURG, 1959). He also started investigations about the effect of Simetone (see below) on photosynthesis (VAN STEEKELENBURG, 1961), but had to stop this work in 1961.

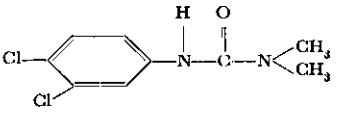
Recently, the subject was taken up by the first author, who confirmed the results about Simetone (VAN STEEKELENBURG, 1961) and extended them. The results described in the following sections are based upon this recent work.

The substituted ureas and the symmetrical triazines are two special and important groups of herbicides. These compounds are taken up by the roots, transported via the xylem and accumulated in the leaves, where they inhibit photosynthesis. The best known compound of the triazine-group is Simazine: 2-chloro-4, 6-bis-(ethylamino)-s-triazine. The solubility of Simazine in water is very low, viz., 5 ppm. According to BISHOP (1962) and others, all amino-triazines have the same effect on photosynthesis. Therefore, Simetone has been taken for our investigations: 2-methoxy-4, 6-bis-(ethylamino)-s-triazine. The solubility of Simetone in water is better, viz., 3200 ppm at 20°C.

As a representative of the substituted ureas, DCMU has been taken: 3-(3, 4-dichlorophenyl)-1,1-dimethylurea.



Simetone



DCMU

The aim of this study was to investigate the influence of Simetone and DCMU on photosynthesis. Measurements have been made with the Warburg-technique, using suspensions of unicellular algae, *Scenedesmus* sp.

Material and methods

The algae (*Scenedesmus* sp.) were cultured as follows: an inoculum, taken from an agar slant, was suspended in 5 ml sterilized culture medium. With a sterilized pipet 0.5 ml of this suspension was brought into a one litre erlenmeyer, containing 250 ml culture medium, which per litre contained:

a. KNO_3	2.5 g	b. KH_2PO_4	0.135 g
c. $MgSO_4$	0.50 g	d. $FeSO_4$	0.006 g
		Na-citrate	0.004 g
		EDTA	0.002 g

In addition, 2 ml of an A4 and B7 solution of trace elements according to Arnon (1938) were supplied. Concentrated stock solutions of the salts mentioned above, were sterilized separately (a, b and c), or in a mixture of suitably matched quantities (d).

The Erlenmeyer flasks were placed on a shaking machine and illuminated from below with fluorescent tubes. Room temperature was 22°C. The suspension was flushed with a stream of air containing 5% CO_2 through a capillary tube closed by a cotton plug. After 4–6 days, the cells were used for an experiment. Cell density then was 3–6 mm^3 cells/ml.

The measurements of photosynthesis were made with a Warburg-apparatus, using six manometers simultaneously (Umbreit, Burris and Stauffer, 1951).

All experiments were carried out at a temperature of 25°C $\pm$ 0.1. For an experiment the cells from a flask, taken from the shaking machine, were centrifuged, washed once and suspended into Warburg-buffer no. 9 (15 ml 0.1 M Na_2CO_3 + 85 ml 0.1 M $NaHCO_3$). Cell density was determined as wet cell volume at dense packing, obtained with Tromsdorff-tubes after 10 min centrifugation at 3000 rpm. Cell density was 3 mm^3 cells/ml, unless stated otherwise.

The vessels in the Warburg-apparatus were ulliminated from below with incandescent lamps (Philips Attralux S-24 V, 150 W). Unless stated otherwise, light intensity was 26×10^4 erg/cm^2 sec., measured at the bottom of the vessels.

For an experiment 10 ml cell suspension was pipetted into each vessel; the herbicide was introduced into the side-arm. Vessels were equilibrated for 15 min and readings taken in 5-min intervals.

Experimental results

a. Inhibition of photosynthesis at different concentrations of the herbicides

First oxygen evolution was measured; then the herbicide was added to the suspension, and after 30, 60 and 90 min oxygen evolution was measured again. The inhibition was expressed in percent, viz.,

$$\frac{\text{ml } O_2 \text{ before addition} - \text{ml } O_2 \text{ after addition}}{\text{ml } O_2 \text{ before addition}} \times 100$$

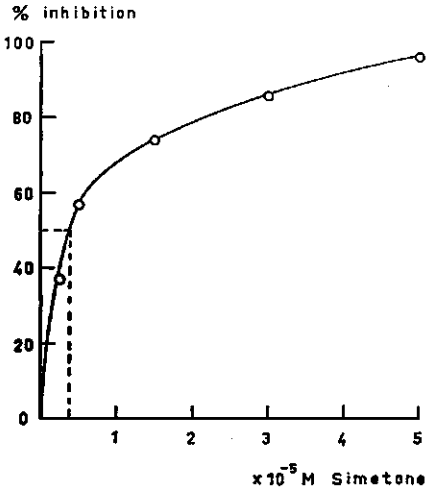


FIG. 1. Effect of different concentrations of Simetone on O_2-evolution; 30 min after addition.

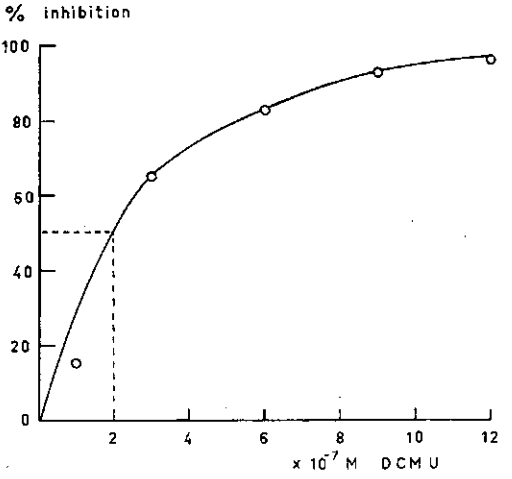


FIG. 2. Effect of different concentrations of DCMU on O_2-evolution 30 min after addition.

Photosynthesis is inhibited for 50% at a concentration of 4×10^{-6} M Simetone (fig. 1). For DCMU this value is 2×10^{-7} M (fig. 2). 60 and 90 min after addition of the herbicide, the inhibition is the same as after 30 min.

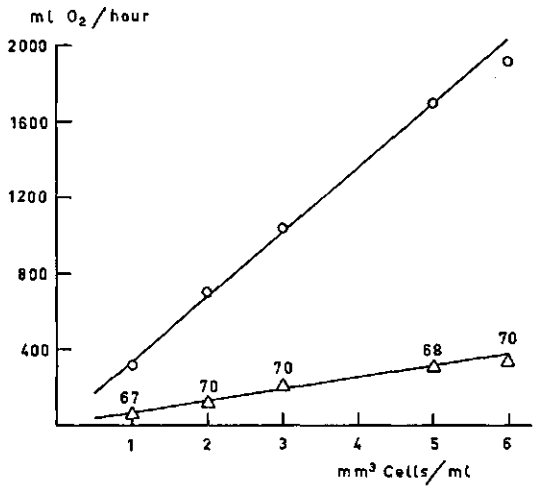


FIG. 3. Effect of 4×10^{-6} M Simetone on O_2-evolution at different suspension densities. ○——○ = before addition of the herbicide; △——△ = after addition. Numbers in lower line: % inhibition.

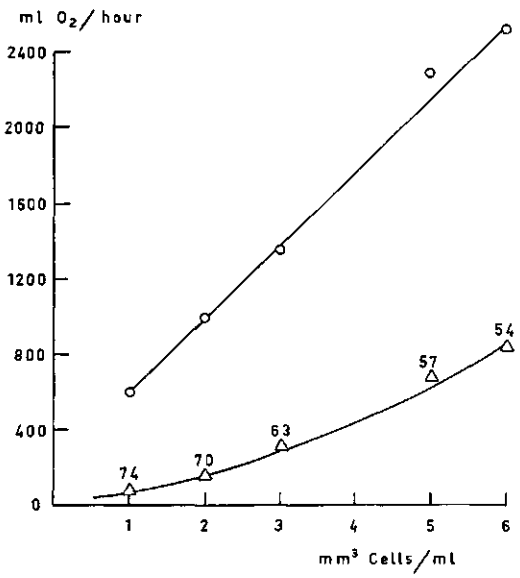


FIG. 4. Effect of 2×10^{-7} M DCMU on O_2-evolution at different suspension densities. ○——○ = before addition of the herbicide; △——△ = after addition. Numbers in lower line: % inhibition.

b. Inhibition at different suspension densities

O_2-evolution was measured at five different suspension densities, and 30 min after the addition of the herbicide, measurement of O_2-evolution was again started.

For Simetone, the inhibition percentages at different suspension densities are nearly equal, suspension density, thus, has no influence on the rate of inhibition of photosynthesis by Simetone (fig. 3). For DCMU, the inhibition percentage becomes lower at higher suspension densities (fig. 4).

c. Removal of inhibition by washing

After estimating the influence of the herbicide upon the O_2-evolution, the cells were centrifuged and washed twice with tap water. Hereafter, they were suspended in fresh buffer and the O_2-evolution was measured again. After the washing the suspension density was made up the same as before.

It is evident, that the inhibition of both herbicides can be removed by washing (fig. 5, 6).

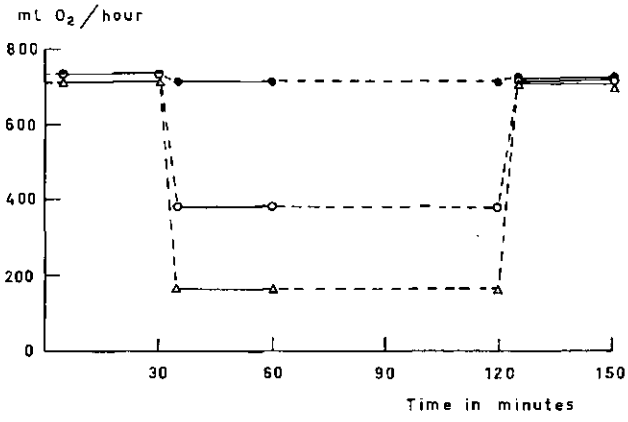


FIG. 5. Removal of the Simetone-inhibition by washing.
●——● = control;
○—○ = 0.25×10^{-5} M;
△—△ = 1×10^{-5} M.

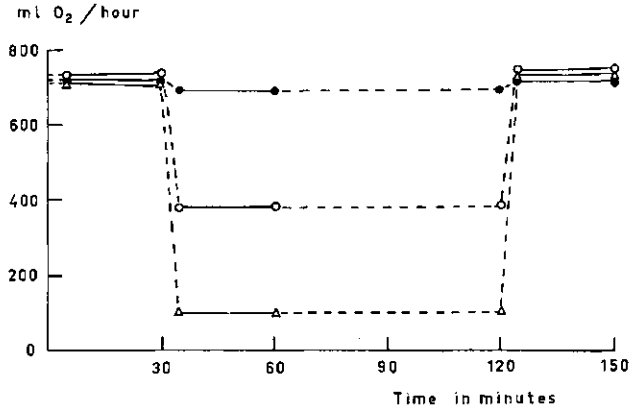


FIG. 6. Removal of the DCMU-inhibition by washing.
●——● = control;
○—○ = 1×10^{-7} M;
△—△ = 3×10^{-7} M.

d. Inhibition at different light intensities

Both Simetone and DCMU have their most pronounced effect in the light-limited range of the curve, but also the temperature dependent portion is strongly affected (fig. 7, 8).

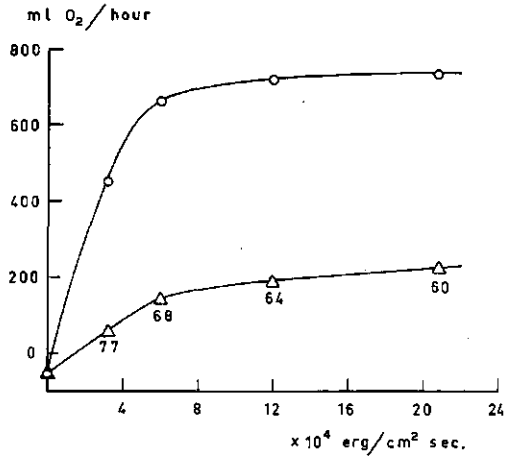


FIG. 7. Effect of 4×10^{-6} M Simetone on O_2-evolution at different light intensities. ○——○ = before addition; △——△ = after addition. Numbers in lower line: % inhibition.

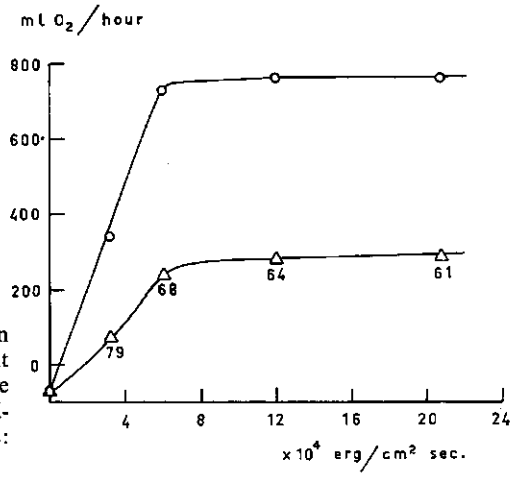


FIG. 8. Effect of 2×10^{-7} M DCMU on O_2-evolution at different light intensities. ○——○ = before addition; △——△ = after addition. Numbers in lower line: % inhibition.

DISCUSSION

Simetone inhibits photosynthesis for 50% at a concentration of 4×10^{-6} M, when photosynthesis is light-saturated (fig. 1). For DCMU this value is 2×10^{-7} M (fig. 2). This agrees with the results of BISHOP (1962) and WESSELS and VAN DER VEEN (1956). The rate of inhibition remains constant 30, 60 and 90 minutes after addition of the herbicides.

In the suspension density experiments so much light is given, that photosynthesis is light-saturated also at the highest suspension density. For the absorption of the herbicide there are two possibilities:

1. The cells accumulate the toxic compound. At equal herbicide concentration, the inhibition percentage then will tend to be lower at higher suspension densities.

2. The cells do not accumulate the toxic compound. In this case, the inhibition percentage will tend to be the same at different suspension densities.

For Simetone, the inhibition percentages at different suspension densities are nearly equal (fig. 3). This is an indication, that Simetone behaves according to possibility 2. Simetone is easily washed out (fig. 5); a possible binding should be a loose one.

For DCMU the inhibition percentages are lower at higher suspension densities (fig. 4), which indicates that the cells accumulate DCMU (possibility 1). Yet, a possible binding of DCMU also should be a loose one, for also DCMU is easily washed out (fig. 6).

Both Simetone and DCMU have their most pronounced effect in the light-limited range of photosynthesis (fig. 7, 8). This is an indication that they act on a photochemical reaction. Since there is also an effect in the temperature dependent portion of the curve, they appear to be active also in a ratedetermining-dark step.

The investigation is being continued.

Summary

The effect of the herbicides Simetone and DCMU on photosynthesis was studied on suspensions of unicellular algae (*Scenedesmus* sp.). Measurements were made with a Warburg-apparatus at a temperature of 25 °C. The suspension medium was Warburg-buffer no. 9.

Simetone inhibits photosynthesis for 50% at a concentration of 4×10^{-6} M; for DCMU this value is 2×10^{-7} M. This inhibition stays constant for a long time after addition of the toxic compound to the cells.

Suspension density does not influence the rate of inhibition by Simetone, which points to an easy equilibration of concentrations in- and outside the cells. For DCMU, at higher suspension densities the rate of inhibition decreases, this points to a high degree of accumulation by the cells. Both Simetone and DCMU are easily washed out of the cells.

Both herbicides have their most pronounced effect in the light dependent part of the photosynthesis-light curve, however the temperature dependent portion is affected too.

Acknowledgements

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