# The Role of Light and Oxygen in the Action of the Photosynthetic Inhibitor Herbicide Monuron

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Monuron treatment of detached flax cotyledons caused a rapid inhibition of $CO_2$ fixation in darkness and light. Chlorophyll breakdown was promoted by increased light intensity. Ethane generation, an indicator of membrane damage, increased as the chlorophyll level decreased. The carotene pigments were destroyed more rapidly than the xanthophylls and chlorophylls. Treatment of cotyledons with monuron in the absence of oxygen almost prevented carotenoid and chlorophyll loss. The addition of the singlet oxygen quencher DABCO delayed chlorophyll breakdown.

The results are discussed in relation to a role for singlet oxygen in the herbicidal action of monuron.

## Introduction

It is well established that many herbicides, including the ureas, uracils, hydroxybenzonitriles, triazines and triazinones, have a primary site of action in the chloroplast [1]. This site is believed to be a proteinaceous component of the thylakoid membrane, and herbicide binding causes the interruption of electron flow between Q and plastoquinone which consequently leads to an inhibition of $CO_2$ fixation [1–3]. Early work with these so-called photosynthetic inhibitor herbicides suggested that plant death was due to starvation [4, 5], however this assumption was questioned when a lack of apparent toxicity was found if herbicide treated plants were maintained in darkness [6, 7]. A greater degree of phytotoxic response was observed when the light intensity was increased [8, 9] and this was linked to wavelengths absorbed by the chloroplast pigments [6]. More recent work has suggested that a primary action of these herbicides is by the redirection of the pigment absorbed excitation energy to initiate cellular deteriorative processes [10–12].

In this paper we report some effects of light and oxygen on the action of the herbicide monuron.

## Materials and Methods

Flax (*Linum usitatissimum* var. Reina) seedlings were grown on moist vermiculite at 25 °C under a continuous illumination of 5.25 W/m². Detached 7 day old cotyledons were floated on water or herbicide solution as appropriate in darkness or light of 5.25 or 30 W/m².

Carbon dioxide exchange was measured by using an infra-red gas analyser (Grubb Parsons Limited). The cotyledons were contained within a small glass sample chamber maintained at constant temperature and humidity. Illumination was provided by a photoflood lamp giving 115 W/m² in the sample chamber.

Chlorophyll was extracted into 80% acetone by grinding a known weight of cotyledons with sand in a mortar and the concentration determined by the method of Arnon [13]. The carotenoid content was measured by a modification of the method of Bishop and Wong [14]. Following extraction into 80% acetone, pigments were partitioned into diethyl ether with the aid of saturated NaCl solution. The ether was taken to dryness and the pigments were taken up in petroleum ether (60–80 °C), and a known volume was streaked onto a silica gel (Kieselgel G nach Stahl, Typ 60), t.l.c. plate which was developed for 45 minutes in petroleum ether : isopropyl alcohol : water (100 : 10 : 0.5). The major carotenoids were resolved in the order $\alpha$- and $\beta$-carotene; lutein and zeaxanthin; violaxanthin and neoxanthin, and were quickly removed and eluted in petroleum ether (carotenes) or ethanol (xanthophylls). The concentration was determined spectrophotometrically at their maximum absorption wavelength using the appropriate extinction coefficients (Jeffrey [15]).

For the determination of ethane, cotyledons were treated in "Medical Flat" bottles sealed with "Suba Seal" bungs. The bottles were laid on the flat side

*Abbreviations:* DABCO, 1,4-diazobicyclo(2,2,2)octane; Monuron, 3-(4-chlorophenyl)-1,1-dimethylurea.

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and samples of air were removed by syringe and assayed for ethane by g.l.c. using a "Pye Unicam G.C.D. Chromatograph" with an alumina column at 125 °C.

## Results

Treatment of flax cotyledons with $1 \times 10^{-3}$ M monuron caused a rapid inhibition of $CO_2$ fixation and this was similar if the cotyledons were incubated in darkness or in light of 5.25 or 30 W/m² (Table I). In contrast, there were considerable differences in the rate of chlorophyll breakdown with the three treatments in the presence of monuron (Fig. 1 B). With light of 30 W/m² complete destruction had occurred by 96 h in contrast to a loss of only 40% with 5.25W/m². When the cotyledons were maintained in darkness a small decrease of only 15% chlorophyll was evident after 144 h treatment.

Table I. $CO_2$ fixation in flax cotyledons incubated with $1 \times 10^{-3}$ M monuron in the dark or light of 5.25 and 30 W/m².

| Incubation Time [min] | μmol $CO_2$/g.F.Wt./h | | |
|---|---|---|---|
| | Dark | 5.25 W/m² | 30 W/m² |
| 0 | 68.0 | 68.0 | 68.0 |
| 15 | 18.6 | 11.1 | 15.4 |
| 30 | 3.2 | 3.5 | 2.2 |
| 60 | 2.3 | 1.9 | 0.9 |
| 120 | 0.0 | 0.0 | 0.0 |

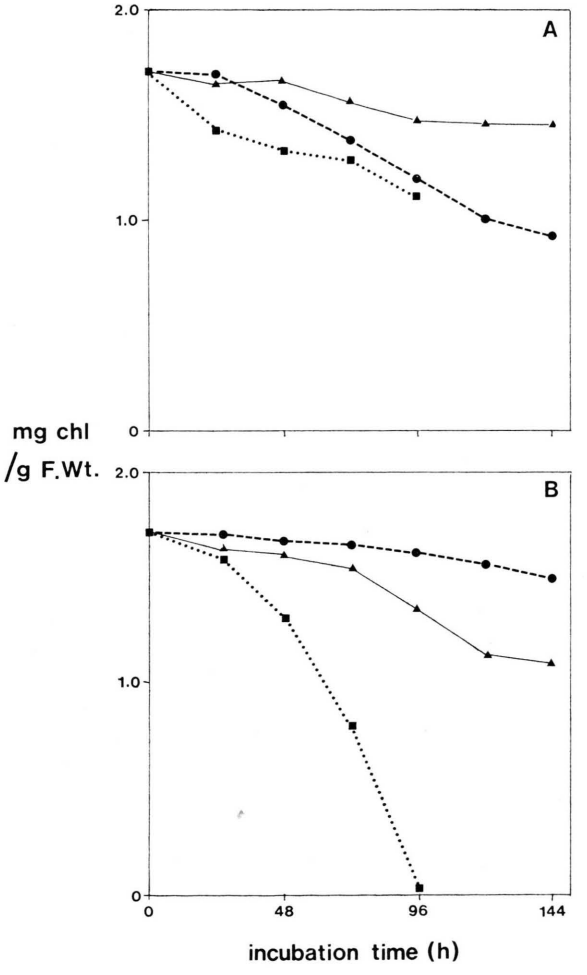


Fig. 1. The chlorophyll content of flax cotyledons incubated on water (A) or $1 \times 10^{-3}$ M monuron (B) in the dark (●-●) or light of 5.25 (▲—▲) or 30 W/m² (■··■).

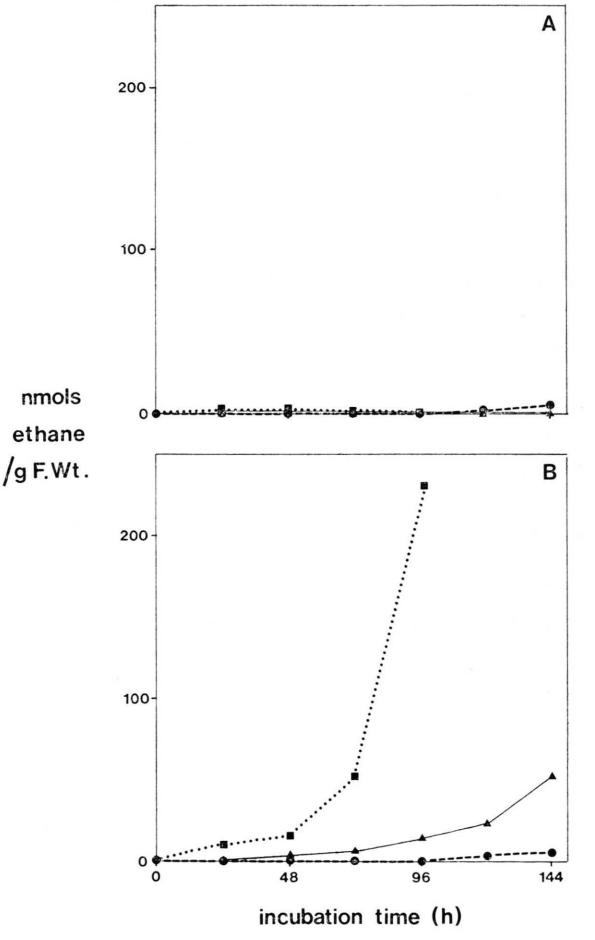


Fig. 2. Ethane generation from flax cotyledons incubated with water (A) or $1 \times 10^{-3}$ M monuron (B) in the dark (●-●) or light of 5.25 (▲—▲) or 30 W/m² (■··■).

Ethane generation, an indicator of lipid peroxidation [16] paralleled the chlorophyll breakdown (Fig. 2 B). After 96 h incubation at 30 W/m², 230 nmol of ethane per gram fresh weight had been evolved from monuron treated cotyledons in contrast to 51 nmol from those incubated under light of 5.25 W/m². There was a minimal evolution of ethane from cotyledons maintained on monuron in total darkness, or from those incubated in the absence of the herbicide (Fig. 2 A).

Table II shows that carotenoid pigment breakdown, and in particular of α- and β-carotene, appeared to precede that of chlorophyll and of the xanthophyll pigments lutein, zeaxanthin, violaxanthin and neoxanthin. When air was replaced by argon in the incubation flasks, the breakdown of all pigments was minimal (Table II).

Table II. The pigment of flax cotyledons at 0 h (7 days old) and after 72 h $1\times10^{-3}$ M monuron treatment in air or argon.

| | mg pigment/g.F.Wt. | | |
|---|---|---|---|
| | 0 h | Monuron/air | Monuron/argon |
| Chlorophyll | 1.669 | 0.956 | 1.620 |
| α and β-carotene | 0.066 | 0.015 | 0.061 |
| Lutein and zeaxanthin | 0.132 | 0.075 | 0.127 |
| Violaxanthin | 0.069 | 0.031 | 0.063 |
| Neoxanthin | 0.048 | 0.015 | 0.043 |

Leaves were treated under a light intensity of 30 W/m². In Erlenmeyer flasks which could be flushed with air argon. Herbicide solutions were degassed before use.

Table II shows the effect of the singlet oxygen quencher DABCO, when added to the aqueous incubation medium. Although there was a limited amount of chlorophyll breakdown with DABCO in the absence of monuron, the quencher retarded the degree of chlorophyll breakdown in its presence.

Table III. The chlorophyll content of flax cotyledons incubated with $1 \times 10^{-3}$ M monuron or $1 \times 10^{-3}$ MDABCO for 96 h.

| Treatment | mg Chlorophyll/g.F.Wt. |
|---|---|
| Water | 1.735 |
| Monuron | 1.184 |
| DABCO | 1.610 |
| Monuron plus DABCO | 1.598 |

Leaves were incubated under light of 5.25 W/m² for 96 h. DABCO was made up in aqueous solution and added to the incubation medium.

## Discussion

The initial action of monuron in inhibiting electron transport and thus $CO_2$ fixation was rapid, and complete within 120 minutes irrespective of the light or dark incubation conditions (Table I). This suggested that the uptake and binding of the herbicide to the chloroplast site was independant of light.

The main visible symptom of herbicide action, chlorophyll loss, was dependant upon light and was greatly increased by increased illumination (Fig. 1). The role of light in promoting pigment destruction was clearly related to an inhibition of electron transport. (Compare Fig. 1 A with Fig. 1 B.) It is generally agreed that one of the functions of carotenoid pigments in the chloroplast is to act as photoprotectors in addition to a role as light harvesting pigments [12, 17]. Photoprotection could take the form of direct energy transfer from excited chlorophyll pigments or indirectly as quenchers of singlet oxygen [18, 19]. The preferential breakdown of certain of the carotenoid pigments especially α- and β-carotene before chlorophyll, would indicate that they were acting in this capacity.

The promoting action of oxygen in chlorophyll breakdown was clearly evident in experiments in which monuron treated leaves were incubated under air or argon (Table II). The absence of oxygen led to a considerable retardation of both chlorophyll and carotenoid breakdown, which suggested that the photoprotective systems were not overloaded. In the presence of air however, excited triplet chlorophyll could interact with triplet (ground state) oxygen to generate singlet oxygen and this appeared to overload the protective system and lead to a rapid carotene and chlorophyll breakdown. A degree of protection was afforded by the use of the singlet oxygen quencher DABCO (Table III).

There is good evidence that singlet oxygen, among other deleterious actions, interacts directly with unsaturated fatty acids in membrane lipids to produce lipid hydroperoxides [20] thus initiating a chain reaction which leads to extensive membrane damage [21]. The monuron and light induced promotion of ethane efflux in parallel to chlorophyll loss, indicated that this could be occuring.

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