Use of Chlorophyll Fluorescence Induction Kinetics to Study Translocation and Detoxication of DCMU-Type Herbicides in Plant Leaves

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Transient levels of the fluorescence induction rise were used to quantify partial photosynthesis inhibition by DCMU-type herbicides in whole leaves. Assays in different crop or weed species showed a good accuracy in measurements (generally, variation was lower than 5%). This technique was applied to the problem of varietal selectivity of wheat towards chlortoluron.

Introduction

Time-dependent variations in chlorophyll fluorescence intensity, at the onset of illumination of a dark-adapted photosynthetic material, were first observed by Kautsky [1] as a transient fluorescence increase followed by a slow decline. The fluorescence rise follows biphasic kinetics, which has been explained [2] by a photochemical reduction of two consecutive electron acceptors of PS II, the primary acceptor Q_A (photochemical phase, O → I), and the plastoquinone pool A or PQ (thermal phase, I → P). Thus, the kinetics of fluorescence induction, reflecting the Q_A^-/Q_A ratio, can be used for probing the oxido-reduction state of PS II centers, although non-redox factors also influence fluorescence emission [3, 4]. Inhibitors acting like DCMU prevent the electron transfer from Q_A^- to a secondary acceptor Q_B [5], without affecting the reduction of Q_A. This results in an increase in the corresponding photochemical fluorescence rise (O → I) up to a maximum constant level.

DCMU-type inhibitors are widely used herbicides such as substituted ureas and triazines (for a review see [6]). The suppression of the slow fluorescence decline (P → S) by these compounds, observable by visual inspection, infra-red photography [7, 8] or simple measuring devices, has been used for detecting plants resistant to triazines [9–11], or monitoring translocation [12] and detoxication [9, 13] of herbicides.

Abbreviations: DCMU, N-(3,4-dichlorophenyl)-N′-dimethylurea; chlortoluron, N-(3-chloro-4-methyl-phenyl)-N′-dimethylurea; PS, photosystem.

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However, the P → S decline is a complex phenomenon, under the dependence of redox and non-redox factors [3, 4], which may themselves be affected by the herbicide treatment. It proved difficult to obtain reliable quantitative parameters from the P → S phase in leaves. To this purpose, the terminal fluorescence intensity (T), measured on a constant area in preilluminated leaves, provides a good accuracy [14]. Another way to quantify PS II inhibition by DCMU-type herbicides in leaves consists in taking advantage of the first phases (O → I → P) from the fluorescence rise, which reflect essentially the $Q_A \rightarrow Q_B \rightarrow PQ$ electron transfer [15, 22].

The differential tolerances of wheat cultivars towards chlortoluron has been explained by slight differences in the rates of biochemical breakdown of this compound [16, 17]. Detoxication, which results either from herbicide breakdown or from other inactivation processes, was also investigated through restoring of the I → P fluorescence phase. Detoxication occurred in tolerant or medium tolerant cultivars, whereas it was almost not observed in susceptible cultivars [15, 17, 19, 20].

We used essentially the wheat/chlortoluron system to evaluate the possibilities provided by the fluorescence rise transients in studying herbicides detoxication processes. Then, we examined the relevance of fluorescence observations.
susceptible cultivars [15, 17, 19, 20].

Materials and Methods

Plants material

Plants were grown on a "perlite" support impregnated with a Hewitt nutritive solution, in growth chambers, at 18 °C (wheat) or 20 °C (weeds) and

16 h illumination. They were transferred to a liquid nutritive solution 1 day before a 24 h treatment in a herbicide solution (one-leaf stage for wheat).

Fluorescence measurements

A 150 watt Zeiss-Perkeo slide projector, with a DC power supply, illuminated a screen with a 25 mm × 7 mm vertical slit, at 10 cm, through a broad band blue filter (400–600 nm) and a Compur photographic electrically triggered shutter. Front fluorescence emission was collected by an optical fiber at a 30° incidence from the illuminating beam, and conveyed to a photomultiplier through complementary red filters (Wratten n° 70, > 620 nm). Whole plants were placed behind the screen and leaves were gently applied against the slit. Plants were maintained in the dark for at least 2 hours before measurements.

Signals analysis

The fluorescence signal was digitalized by an analogic/digital 12 bits conversion card and was stored as 10000 × 1 ms channels, in an Apple II microcomputer, using an Assembly programm. Then, it was analyzed using a Basic programm. Channel 1 was defined as the first detection of fluorescence signal. Shutter opening time was less than 2 ms (2 channels), so that O level, corresponding to channel 2, was estimated from signal values at channels 3 and 4. I level corresponded to a mean value computed in a adjustable time domain (usually 50 to 70 ms), defined from the signal shapes in control and herbicide treated leaves. P level was detected, over an adjustable time domain, as the maximum value obtained by successively comparing 100 channels-mean values. These values were used to compute the ratios P/O, I/O, I/P, (I−O)/(P−O) and the half-time of the photochemical rise (O → I). Approximately 100 measurements per hour were possible.

Chloroplasts, were prepared as previously reported [9]. They were ruptured by a hypotonic shock. The same medium was used for extraction and fluorescence measurements: 0.4 M sucrose, 50 mM Tricine (pH 7.8), and 10 mM KCl, 2 mM $MgCl_2$.

Results and Discussion

Table 1 shows examples of mean values and coefficient of variation of ratios calculed between OIP levels of the fluorescence rise. These ratios displayed little intraspecific and interspecific variations in healthy plants. In our conditions, no dip D [3] was observed.

Table I. Mean values and coefficients of variations ($\sigma/\bar{x}$) of ratios between O, I, P levels of fluorescence rise. For I/P and (I−O)/(P−O), C.V. were computed for the complementary variable I−$\bar{X}$. a: upper face of leaf. b: lower face.

	P/O	I/O	I/P	(I−O)/(P−O)
Wheat[a]	5.97	2.09	0.35	0.22
	9.9%	8.7%	4.2%	3.5%
Barley[a]	5.94	2.08	0.35	0.22
	9.2%	8.9%	4.0%	4.0%
Vicia faba[a]	5.67	2.36	0.42	0.29
	7.5%	6.8%	3.4%	3.0%
Avena fatua[a]	4.45	1.79	0.40	0.23
	12.8%	6.4%	9.0%	9.5%
Centaurea cyanus[a]	6.09	2.36	0.40	0.27
	15.0%	10.4%	9.3%	6.4%
Sinapis arvensis[a]	6.41	2.22	0.35	0.22
	8.4%	9.0%	1.9%	2.3%
Sinapis arvensis[b]	6.40	2.64	0.41	0.30
	12.0%	9.6%	4.6%	3.8%

P/O was the most variable parameter and appeared to depend on physiological conditions of plants. Values lower than 4.5 were generally an indication of bad growing conditions for a particular species. After 2 h dark adaptation, P/O was still increasing, until 8–10 hours in the dark (typically 5 to 7), then it slowly decreased. These variations between the relative magnitudes of variable fluorescence (O → I → P rise) and constant fluorescence (O level) also affected I/O and, to a lesser extent, I/P ratios. (I−O)/(P−O), which relates the photochemical rise to variable fluorescence, without taking into account constant fluorescence, proved to be the most accurate parameter. It remained constant from 2 h until 15 h in the dark (Fig. 1 b). In the case of wheat, coefficients of variations for this parameter were always lower than 4% and reached 1.5% with some particular batches of plants. The accuracy of the measuring system, independently of plant material variations, was checked using suspensions of chloroplasts, and was found to be 0.9%. With dicotyledons, (I−O)/(P−O) values were slightly higher in the lower face of the leaves [18]. This was not observed with monocotyledons.

Thus, in order to measure the increase of the photochemical rise (O → I) induced by DCMU-

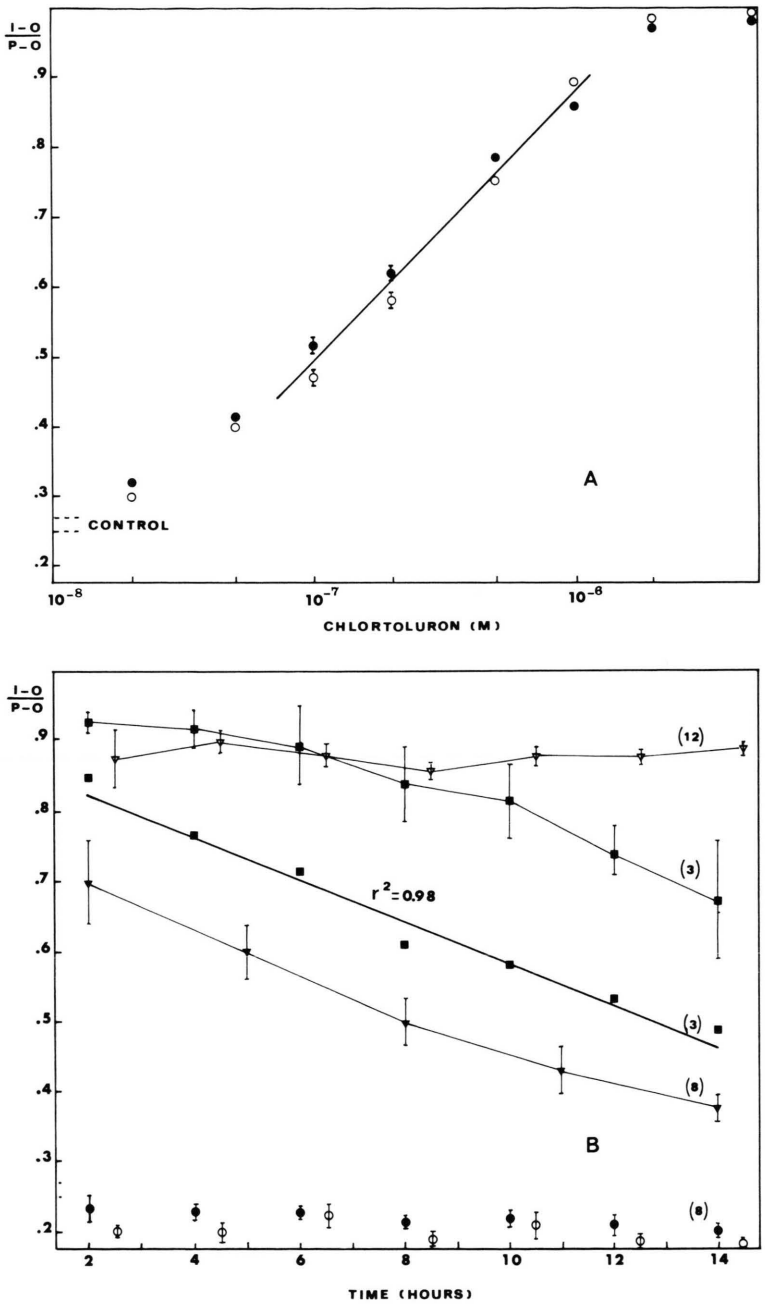

Fig. 1. a) Effect/concentration curve of chlortoluron in wheat chloroplasts (5μg/ml chlorophyll). (●) tolerant; (○) susceptible.
b) Evolution of photochemical phase after 2 h dark adaptation. End of treatment at 0 h, 18 °C. (●) Tolerant and (○) susceptible, untreated; (▲) tolerant, 0.5 ppm 24 h chlortoluron; (■) two samples of tolerant, 1 ppm 24 h chlortoluron; (▽) susceptible, 0.5 ppm 24 h chlortoluron; (n) number of replicates.

type herbicides, the (I−O)/(P−O) ratio was used. However, the I/P ratio may prove useful, since I and P levels are detectable with a fast-response recorder [15].

In leaves treated at partially inhibiting herbicide concentrations, blocked and non-blocked PS II centers coexist. However, PS II units are not independent [4]. This results in a non-linear relation between (I−O)/(P−O) and Q_A^-/Q_A. Furthermore, P/O ratios were decreased in herbicide-treated leaves, in our conditions of light excitation. This could be ascribed to a quenching by PQ [21]. Values of (I−O)/(P−O) observed in leaves can be refered to values measured on isolated chloroplasts at dif-

ferent herbicides concentrations (Fig. 1 a). The half-inhibitory concentration thus determined corresponded to that obtained in Hill reaction [22]. The fluorescence effect/concentration in chloroplasts curve allows an estimation of the relative variations in herbicide concentrations from $(I-O)/(P-O)$ variations in the leaves. However, from Fig. 1, it is not possible to deduce total herbicide concentrations (on a fresh weight basis) in the leaves. PS II inhibitors are hydrophobic compounds which partition into membranes, or can be adsorbed by biological macromolecules. It was observed that suppression of I → P phase occurred at 10-fold higher herbicide concentrations in leaves than in a chloroplast suspension [17, 23].

After a root-applied herbicide treatment (24 h, with 16 h illumination), PS II inhibition was first detected in the lower part of wheat leaves. Then, after placing plant roots in an untreated medium, the absorbed herbicide was translocated towards the top of the leaf, driven by the transpiration stream, so that photosynthesis recovery was first observed in the lower part. In order to study detoxication without interference of translocation, plants were maintained in the dark, in a water saturated atmosphere. Reduction of translocation was controlled by a 24 h herbicide treatment in the dark. After a 2 h dark adaptation, measurements had to be performed within 12 h, since a slow increase of $(I-O)/(P-O)$ was observed at longer times. Fluorescence was always measured on the same part of the leaf.

The dispersion of $(I-O)/(P-O)$ values was greater between leaves in which PS II centers were partially inhibited by herbicide, as compared to untreated plants. This is ascribable to differences in the rates of herbicide translocation. In order to overcome this heterogeneity, the decrease of $(I-O)/(P-O)$ versus time was followed for individual plants. Plants displaying similar PS II inhibition were pooled. A linear relationship was obtained within the 0.85 and 0.50 values of $(I-O)/(P-O)$ (Fig. 1 b), which corresponds to the linear part of the effect/concentration curve on chloroplasts (Fig. 1 a).

This can be expressed in terms of chlortoluron concentration H:

$$(I-O)/(P-O) = A \log H + B. \quad (1)$$

Assuming a first-order kinetic for chlortoluron breakdown in wheat [17],

$$H = H_O e^{-kt}. \quad (2)$$

By replacing H value from (2) in (1), it comes:

$$(I-O)/(P-O) = A t + B'$$

which is in agreement with Fig. 1 b.

In the tolerant variety, $(I-O)/(P-O)$ decreases from 0.82 to 0.47 in 12 h (Fig. 1 b). By reference to Fig. 1 a, this represents a 80% inactivation of chlortoluron after 12 h. These values of photosynthesis recovery obtained by fluorescence appeared fairly constant within tolerant and medium tolerant varieties [15, 19] and were in agreement with results from CO_2 uptake measurements [20].

The behaviour of susceptible varieties contrasted sharply, since almost no I → P recovery occurred [15, 19]. This was also noticed in CO_2 uptake measurements [20]. Differences in detoxication rates of chlortoluron observed by fluorescence between tolerant and susceptible wheat varieties were much greater than those found in the biochemical breakdown of this compound after extraction from leaf tissues [16, 17]. A similar situation occurred with the experimental herbicide U.K.J. 1506 (PUK), acting like DCMU. I → P recovery was greater in barley than in wheat, whereas no differences were found in the concentrations related to fresh weight [23].

In order to detect a possible irreversible effect of chlortoluron at the level of PS II, chloroplasts from herbicide treated wheats were extracted and washed. Immediately after the end of treatment, they displayed the same fluorescence induction than control chloroplasts. From 3 days after treatment an increase of I level could be noticed in the susceptible variety, which corresponded to a decrease in Hill reaction activity, partially relieved by silicomolybdate. The same effects were observed in the tolerant varieties treated at higher doses. Thus, earlier damages by action of a DCMU-type herbicide affect the reducing side of PS II, after a period of total photosynthesis inhibition [24]. However, this cannot explain the differences in recovery showed in Fig. 1 b.

The biologically active fraction of a herbicide which is detected by fluorescence, is much lower than the amount of herbicide present, since part of it is inactive by partition or adsorption in leaf tissues. This is not taken into account by chemical analysis, which could explain quantitative discrepancies observed between photosynthesis recovery and biochemical breakdown of herbicide.

The relevance of fluorescence tests has been confirmed by correlation with varietal susceptibility to

chlortoluron in wheat [15, 19] or interspecific selectivity for some other DCMU-type herbicides (data no shown).

Conclusion

The transients of the fluorescence induction rise provide accurate parameters to quantify partial PS II inhibition by DCMU-type herbicides in plant leaves. In the same time, they provide some control of the physiological state of plant material. Compared to CO_2 or O_2 measurements, fluorescence techniques provide some particular advantages. They are convenient for localized observations in different parts of a leaf. Short measuring times allow a great number of observations and replicates. Fluorescence rise parameters directly reflect the PS II inhibition by herbicides, and reference to curves of effect/concentration in chloroplasts is possible.

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