Activity of Photosynthetic Herbicides in Intact Pea Leaves Measured by Prompt and Delayed Chlorophyll Fluorescence

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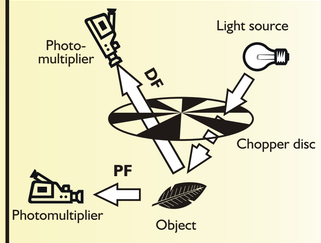
Principle of delayed fluorescence measuring



The actinic light is modulated by a rotating chopper disc to obtain alternating light and dark periods of 5.5 ms each. Prompt fluorescence is detected during the light periods; delayed fluorescence - during the dark periods. [1,2]

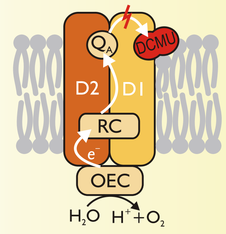
The induction curves are assembled from data points measured in consecutive cycles. The time resolution is thus 11 ms.

Mechanism of action of PS2 herbicides

PS2 herbicides are specific inhibitors of the electron transport. [3-8]

The herbicide binds to the Q_B-site of the D1 protein displacing plastoquinone. As a result the electron flow is blocked between Q_A and Q_B. [5,6]

H-bonds are formed either with Ser_{264} or His_{215} of the D1 protein. DCMU and atrazine are Ser_{264} type while dinoseb is His_{215} type. [7,8]



Materials 1

1. Plant material

Pea plants (*Pisum sativum*, L. cv. Ran-1) were grown as a water culture on a Knop's nutrient solution in a climate chamber at 24-26°C temperature, 65-75% RH and 60 umol.m^{-2}.s^{-1} light intensity. 14-days old plants with 5 expanded leaves were used in all experiments. 3rd leaf was used in experiments with detached leaves. The luminescence was measured from the adaxial leaf surface.

2. Herbicides

- diuron - 3-(3,4-dichlorophenyl)-1,1-dimethylurea (SIGMA)
- atrazine - 2-ethylamino-4-chloro-6-isopropylamino-1,3,5-triazine (SERVA)
- dinoseb - 6-(sec-butyl)-2,4-dinitrophenol (SERVA)

Herbicides were initially dissolved in ethanol to a concentration of 3 mM. Final solutions for treatment contained no more than 1% ethanol.

Methods 2

1. Importing herbicides into the leaves

Leaf diffusion

Detached leaves are put into petri dishes between two layers of filter paper.

Herbicide solution is added to cover the paper.

The leaves are kept submerged for 2 hours at 25°C and illumination of 1800 lx then for 1 hour in the dark.

Infiltration

Detached leaves and herbicide solution are put into a syringe. The air is driven out of the intercellular spaces by applying low pressure. Consecutive high pressure for the solution to fill the leaf tissue.

After infiltration leaves are kept for one hour in the dark.

Stem transport

Whole pea plants are used. Stems are are cut above the root dipped into herbicide solution.

Plants are incubated for 20 h (12 h dark and 8 h at 50 umol.m^{-2}.s^{-1}) at 25°C.

Herbicides are actively transported by the plants. [3]

2. Detection of the herbicide effect

After application of the herbicides leaves are dark adapted for 1 h .

Induction curves of PF and DF are simultaneously measured for a period of 1 min.

Induction parameters are calculated and averaged.

The herbicide activity is assessed by the half-inhibition concentration, estimated from concentration curves for different parameters.

Induction curves 3

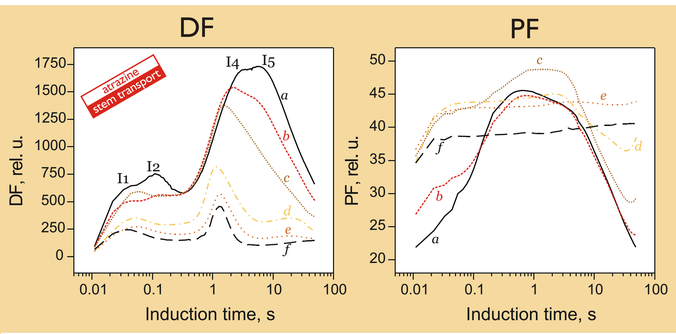


Fig. 1. Induction curves of DF and PF of pea plants incubated for 20 h in different concentrations of atrazine: *a* - control; *b* - 10^{-7} M; *c* - 3.10^{-7} M; *d* - 10^{-6} M; *e* - 10^{-5} M; *f* - 10^{-4} M.

Effects of atrazine applied by stem transport on the DF induction curves:

- **The overall intensity of DF decreases.** DF is driven by the electron transport and the transmembrane potential. [9,10] Both are inhibited by the herbicide.
- **The D2 dip after I2 disappears.** The I2-D2 drop of DF is related to the photoinduced reduction of the PQ pool. [1,2]
- **The I2/I1 ratio decreases.** This ratio reflects the rate of electron transport on the acceptor side of PS2. [1]
- **I5 disappears. I4 decreases but remains distinct even at saturating concentrations.** I4 is generally attributed to the stimulation of DF by the transmembrane gradient. [9] We assume that at high herbicide concentrations I4 is due to a cyclic electron transport around PS1.

Effects of atrazine applied by stem transport on the PF induction curves:

- **The initial fluorescence increases.** In the present setup the first measured point of PF is around 10 ms. The blocking of the electron transport leads to a very rapid rise from Fo to Fp. [11] Hence the apparent (measured) Fo is higher in herbicide-treated samples.
- **The variable fluorescence decreases.** As a result of the Fo rise the maximal variable fluorescence is lower in herbicide-treated plants. Very high concentrations also decrease Fp (static quenching?)
- **The drop of PF after Fp disappears.** In a normal physiological state after reaching its maximum the fluorescence drops again mainly due to reoxidation of the electron pool and energization of the membrane. [11] These quenching mechanisms are eliminated by the herbicide.

Parameters 4

The specific effects on the induction curves can be quantified by various PF and DF parameters.

Parameters calculated from individual induction curves are averaged and presented as a concentration dependence. These "titration" curves are then fitted to a Boltzman sigmoidal function and the concentration of half-inhibition is found (or its negative logarithm, pI50).

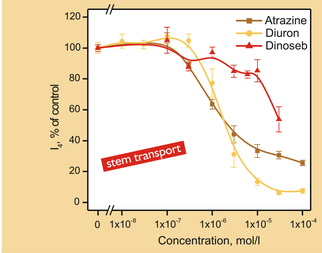


Fig. 2. Concentration dependence of the I4 level of plants incubated for 20 h on a medium containing diuron, atrazine, or dinoseb.

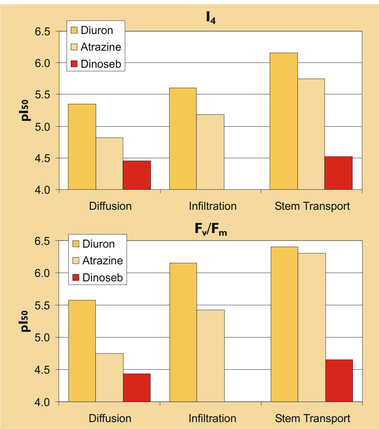


Fig. 3. Comparison of the indices of half-inhibition (pI50) for the three herbides applied to pea plants by diffusion, infiltration or stem transport.

Conclusion 5

The delayed fluorescence induction parameters as well as prompt chlorophyll fluorescence were successfully applied as a quantitative test to estimate the effects of diuron, atrazine and dinoseb in intact leaves. A comparison between the three herbicides for all tested herbicide treatment procedures put diuron as the strongest of the three, followed by atrazine, and dinoseb as the weakest. However, the relative differences between the herbicides were not equal. Atrazine had much lower activity than diuron when applied by leaf diffusion or infiltration and was nearly as strong as diuron when transported through the stems.

The weakest effects were achieved when herbicides were added by leaf diffusion for 2 hours. Vacuum infiltration of leaves induced a stronger herbicide effect but at the expense of high statistical dispersion and poor reproducibility. Stem transport was the most effective method of treatment. The treatment itself did little damage to the sample and the results had low statistical error.

The concentrations of half-inhibition calculated from different parameters were comparable but not the same. The PF and DF parameters are related to a number of photosynthetic processes responding differently to the herbicide action.

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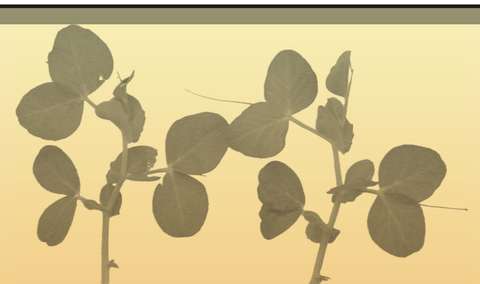

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