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Application of prompt and delayed chlorophyll fluorescence for analysis of the action of photosynthetic herbicides in intact leaves and thylakoid membranes of pea

Dissertation (summary)

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AIM OF THE WORK

Photosynthetic herbicides can be used in the laboratory research for two purposes: 1) to achieve maximal inhibition of the sample in order to study its behaviour when the electron transport is interrupted; 2) to apply a given dose of herbicide and evaluate its effect on the sample. In the latter case, the effect would be dependent on the herbicide concentration in the vicinity of the target site. If the sample is an intact leaf or whole plant, this concentration could greatly differ from the concentration of the treatment solution, depending on what part of it has reached the chloroplasts. Therefore, the method and conditions of treatment can have impact on the measured effect. This makes the measuring of the herbicide activity *in vivo* a difficult task.

The major goal of this work was to investigate the action of the PS2 herbicides atrazine, diuron and dinoseb in intact pea leaves and isolated thylakoid membranes on the prompt (PF) and delayed fluorescence (DF) parameters under different treatment and measurement conditions.

The following experimental tasks were assigned in order to achieve this goal:

1. To analyse the mechanism of action of the Photosystem 2 (PS2) herbicides on the luminescent properties: PF and DF induction curves, parameters of DF decay, dependence of PF and DF on the luminescence potential; to select parameters for quantitative measurement of the herbicide effect in intact leaves.
2. To test and compare different methods for herbicide treatment of leaves and to find the most appropriate method for treatment and evaluation of the herbicide effect; to compare the effects of atrazine, diuron, and dinoseb, applied by the different methods.
3. To investigate the influence of different factors (light intensity, duration of treatment, leaf age) on the measured herbicide activity and to optimize the treatment conditions.
4. To reveal the dynamics of the herbicide effect by registering induction curves and fluorescence images.
5. To investigate the impact of temperature during registration on the luminescence parameters in control and herbicide-treated plants and the dependence of the herbicide effect on temperature.
6. To investigate the influence of pH of the thylakoid membrane suspension on the effects of diuron, atrazine and dinoseb.
7. To develop a computer program, automating the processing and analysis of fluorescence data, gathered with the FL-2006 fluorometer.

MATERIALS AND METHODS

Plant Material

Pea plants were grown for 14 days a water culture on Knop's nutrient solution in a climatic chamber under controlled conditions.

Thylakoid membranes were isolated after Whatley and Arnon (1963) with modifications, frozen and stored in liquid nitrogen before measuring.

Treatment with Herbicides

The following herbicides were used: 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). The herbicides were introduced into the leaves by three different methods:

- Leaf diffusion – detached leaves were kept immersed in herbicide solution for 2 hours under illumination and 1 hour in the dark.
- Infiltration – detached leaves were vacuum-infiltrated using a syringe according to Malkin et al. (1992).
- Stem transport – after removing the roots the stems were dipped in herbicide solution and the plants were kept for 12 hours in the dark and 8 hour under continuous illumination.

Measurement of the Herbicide Effects

- Simultaneous registration of prompt and delayed fluorescence using an FL-2006 fluorometer (Test, Russia)
- Registration of DF dark decay kinetics
- Registration of high resolution PF induction curves (OJIP curves) using a HandyPEA fluorometer (Hansatech Instruments, UK)
- Videorecording of fluorescence images using FluorCam M690 apparatus (PS Instruments, Czech Republic)
- Registration of PF and DF induction curves, DF dark decay curves, and OJIP curves at different temperatures from 5 to 45 °C
- Registration of temperature curves of the steady-state PF and DF levels and steady-state DF dark decays in the interval 5–65 °C

Software for Data Processing

A computer program, Indwin, was designed specifically to automate the processing of the fluorescence data gathered with the FL-2006 equipment. It was developed in Microsoft Visual Basic environment.

SUMMARY OF THE RESULTS

1. The quantity named luminescence potential was derived theoretically and calculated from the experimentally measured values of PF and DF. It revealed that during the first second of induction the changes in PF and DF are associated with photochemical quenching, and the later changes within the first minute – with nonphotochemical quenching.
2. The analysis of the herbicide action on PF and DF induction curves showed that the maxima I_1 and I_4 found in the DF induction curves are dependent on the transmembrane potential, and the maxima I_2 and I_5 are influenced by the electron transport and the redox state of the PS2 acceptors.
3. The effect of PS2 herbicides can be estimated in intact leaves by analyzing PF and DF induction curves. The parameters I_4, F_v/F_p and $1-V_J$ were found as suitable measures of the herbicide effect. The indices of half-inhibition of the corresponding parameters can be used as a measure of the herbicide sensitivity of the samples.
4. The efficiency of the herbicides depends on the method of their application. The comparison between three methods – leaf diffusion, infiltration, and stem transport, showed that the best method for treating leaves with urea/triazine herbicides in terms of effectiveness and consistency is stem transport.
5. The herbicide action depends on the conditions during treatment and on the physiological state of the leaves. The effect was greater in younger leaves, because they predominated in the herbicide uptake. The effect was alleviated by irradiating the samples with moderately high light intensity.
6. The statistical variation of the measured parameters increased significantly in leaves treated with diuron by leaf diffusion. At non-saturating concentrations the frequency distribution was bimodal, suggesting that a certain part of the samples remained unaffected by the treatment.
7. The herbicide transport and distribution within the leaf tissues can be dynamically monitored by chlorophyll fluorescence imaging. The translocation of atrazine goes faster compared to diuron.
8. The PF and DF parameters of untreated leaves and the relative effects of the herbicides on them depend on the temperature of registration. In general, an increase of the herbicide activity was observed around room temperatures.
9. The inhibitory effect of herbicides in isolated thylakoid membranes was modified by pH of the suspending medium. The pH dependence of the effects was similar for herbicides binding to Ser_{264} (diuron and atrazine) as opposed to herbicides binding to His_{215} (dinoseb).