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ARTICLE

Effect of pyrethroid insecticides on the photosynthetic activity of pea mesophyll protoplasts

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ABSTRACT Pyrethroids are plant protecting substances whose effects on treated plants are widely monitored with often conflicting results. Field experiments showed no alteration in photosynthetic rates by a synthetic pyrethroid permethrin (Haile et al. 1999), while other studies showed an inhibition in PSII reactions by the halogen side of the molecules, and suggested Q_A as a target site of interaction, like in the case of urea-type herbicides (Bader and Schuler 1996). In order to clarify these opposing observations, photosynthetic effects of pyrethroids were re-tested.
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KEY WORDS

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microscopy PAM
oxygen evolution

Plant protecting substances, such as insecticides, are supposed to have a negative effect only on target organisms, and should have a good tolerance with respect to the treated plants. Derived from pyrethrins, which naturally occur in the flowers of *Chrysanthemum* species, pyrethroids, such as deltamethrin, permethrin, cypermethrin and fenvalerate, are neurotoxins causing ataxia, tremors and hypersensitivity in both vertebrates and invertebrates. Previous studies have shown that a negative effect of pyrethroid insecticides on plants cannot be excluded. Repeated spraying of field grown *Solanum tuberosum* plants with deltamethrin showed altered morphogenetic development, including elevated chlorophyll and thylakoid content in each grana, smaller starch granules correlating with the lower chloroplast volume, and increased Rubisco activity (Fidalgo et al. 1993). Cypermethrin and fenvalerate were shown to have genotoxic effects as they induce chromosomal breaks and mitotic aberrations in root meristem cells of *Allium cepa* (Chauhan et al. 1986).

Pyrethroid-containing chemicals used in agriculture interact with the photosynthetic electron transport chain, as observed by fluorescence induction curves in intact leaves and oxygen evolution of cell cultures and chloroplasts (Bader and Schuler, 1996). In contrast, 3-year-long field experiments showed unaltered or even increased photosynthetic rates in alfalfa and soybean plants treated with permethrin and other insecticides (Haile et al. 1999).

On the other hand, testing the activity of pure pyrethroids, rather than pyrethroid-containing chemicals applied in the studies above is desirable, since commercially available formulations (e. g. DECIS, Sumicidin, Ambush, Ripcord) contain substances, which facilitate the penetration into the cells (Baeza-Squiban et al. 1987), and may have additional effects on photosynthesis. The potential impact of pure pyrethroids on photosynthesis were re-tested and determined by a microscopy–pulse amplitude modulation (Microscopy-PAM) chlorophyll fluorometer.

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Materials and Methods

Chemicals

Cellulase (Onozuka R-10) and macerozyme (R-10) were from Sigma. Synthetic Pyrethroids (Permethrin, Deltamethrin, Cypermethrin, Fenvalerate; Supelco, USA) were diluted in dimethyl sulfoxide (DMSO) before the addition to the suspension medium. No other solvent or chemical agent was added to the mixture.

Preparation of mesophyll cell protoplasts

Pea (*Pisum sativum* cv. Rajnai törpe) plants were grown hydroponically in the greenhouse at 24-26°C in a modified Hoagland solution. Fully expanded leaves from the upper nodes of 2-3-week-old plants were used in the cell preparations.

Mesophyll protoplasts from pea leaves were isolated from the abaxial epidermis by a one step enzymatic digestion (Riazunnisa et al. 2007) with a slight modification. Following digestion, protoplasts were placed in a suspension medium containing 0.5 M sorbitol, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES (pH = 7.5 with KOH) and 1 mM sodium bicarbonate. Changes in the composition of the suspension medium are indicated at a given experiment.

Estimation of chlorophyll content

Chlorophyll content in protoplast preparations was estimated by extracting into 80% v/v acetone. The absorbance of the

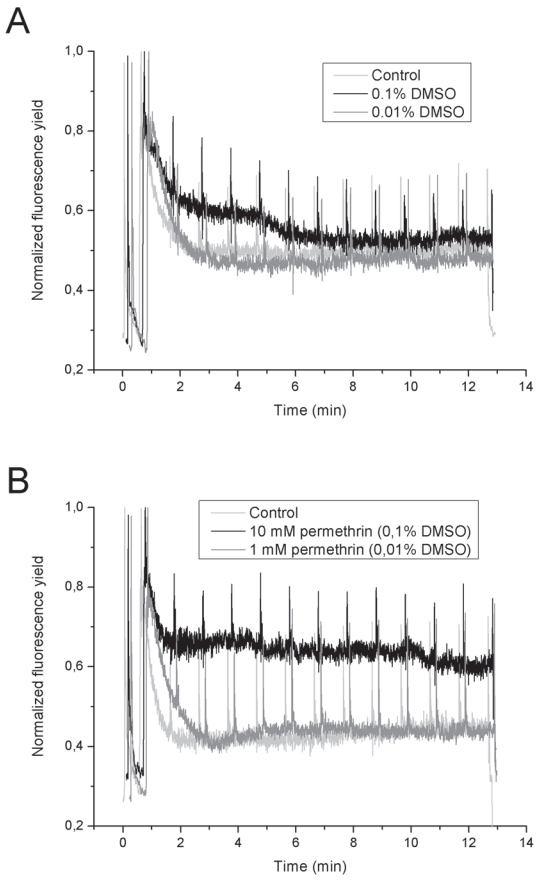

Figure 1. (A) DMSO affects chlorophyll fluorescence of mesophyll cells in 0.1% v/v concentration measured by Microscopy-PAM. (B) 10 mM permethrin (in 0.1% v/v DMSO) increased fluorescence yield of mesophyll cells, which may partly be due to the presence of DMSO (see Fig. 1A), while 1 mM permethrin (in 0.01% v/v DMSO) had no effect. Normalized fluorescence yield curves were plotted with a 5 s shift in time compared to each other for better separation.

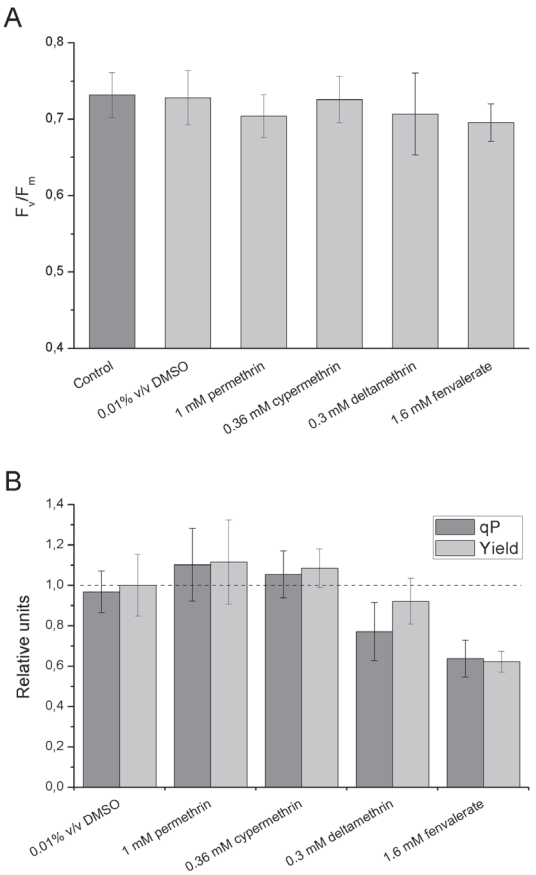

Figure 2. Effects of pyrethroids on photosynthetic parameters of mesophyll cells. Pyrethroids were used in their maximal possible concentration in 0.1% v/v DMSO solvent, which is the threshold amount of solvent-induced effects. (A) Optimal quantum efficiency (F_v/F_m) remains around control values in dark adapted insecticide-treated mesophyll protoplasts. (B) Deltamethrin and fenvalerate decreases photochemical quenching (qP) and effective quantum efficiency (Yield). Steady-state qP and Yield parameters were normalized to their control values (n = 3; ± SD).

acetone extracts were measured at 646.8 and 663.2 nm by a spectrophotometer (Hitachi U3310, Japan) according to the method of Lichtenthaler (1987).

The experimental system

Photosynthesis of single mesophyll protoplasts was monitored by chlorophyll *a* fluorescence using a saturation pulse method (PAM) with a microscopy–pulse amplitude modulation (Microscopy-PAM) chlorophyll fluorometer (Heinz Walz GmbH, Germany) mounted on a Zeiss Axiovert 40 inverted epifluorescence microscope (Zeiss GmbH, Germany). Microscopy-PAM system was made up of a single blue light emitting diode (470 nm) which provided the modulated measuring light as well as actinic light and saturation pulse; a photomultiplier serving as fluorescence detector and a PAM control unit driven by WinControl software.

F_m was obtained by exposing the dark adapted sample to a saturating short pulse (0.8 s). F_v/F_m was calculated according to Genty et al. (1989). Maximum fluorescence values in the light adapted state (F_m') were determined at the end of a 12-min actinic illumination. Steady-state qP ($\Delta F/F_m' - F_o$) was determined according to the method described by Schreiber et al. (1986) where ΔF equals $F_m' - F_s$ and F_s is the steady-state fluorescence yield during actinic illumination. Yield ($\Delta F/F_m'$) was determined according to Genty et al. (1989).

Results and Discussion

Aiming to accurately measure the concentration-dependent effect of pyrethroids on photosynthesis, mesophyll protoplasts were used with easily accessible membranes for chemicals. Pea mesophyll protoplast were kept in dark at 4°C in the experimental solutions and dark adapted on the

microscope stage for 15 minutes before the Microscopy-PAM measurements. In order to achieve better signal/noise ratios, a batch of 5-10 cells were put in the focus of the measuring beam.

Pyrethroids, together with cypermethrin, deltamethrin and fenvalerate were solubilized in DMSO and stock solutions were prepared by the addition of distilled water in 9:1 ratio. Stock solutions were diluted in suspension medium and added to protoplasts in different concentrations indicated at the given experiment. In order to check whether the solvent DMSO modifies the photosynthetic activity of the cells, fluorescence induction curves recorded in protoplasts placed in 0 (control), 0.1 and 0.01% v/v DMSO solutions were compared (Fig. 1A). The results showed that DMSO in 0.1% v/v increased the fluorescence yield, decreased the optimal quantum efficiency (F_v/F_m), photochemical quenching (qP) and effective quantum efficiency (Yield) parameters, but had no effect in 0.01% v/v concentration. This result set the highest pyrethroid concentrations to be used in further experiments. For instance, the permethrin solution used in 10 mM concentration increased the steady-state fluorescence, but was excluded because of its disturbing DMSO content of 0.1% v/v (Fig. 1B).

Application of permethrin and cypermethrin in a maximal concentration allowed by a non-disturbing DMSO content in the suspension medium, in agreement with previous results of Bader and Schuler (1996), showed no appreciable impact on fluorescence parameters (Fig. 2). Although F_v/F_m remained around the control values in every treatment (Fig. 2A), Yield and qP decreased by deltamethrin and fenvalerate applied in 0.3 and 1.6 mM concentrations, respectively. Oxygen evolution measurements conducted in mesophyll suspensions also confirm these results (data not shown).

While these pyrethroid contents in plant tissues are extremely high compared to agricultural applications, phytotoxicity toward given plant species in low concentrations used in agriculture cannot be excluded.

Acknowledgement

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