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Photosynthesis and growth responses of grapevine to acetochlor and fluoroglycofen

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ABSTRACT

Acetochlor and fluoroglycofen are herbicides used in vineyards to eradicate weeds. This present study characterized the effects of these chemicals on photosynthetic characteristics and the antioxidant enzyme system in non-target grape leaves. The results showed that acetochlor and fluoroglycofen reduced net photosynthetic rate in a dose-dependent manner, but also reduced or increased pigment contents, respectively. According to chlorophyll fluorescence measurements, acetochlor and fluoroglycofen decreased the photochemical efficiency of photosystem II in the light and increased non-photochemical quenching. These herbicides enhanced malondialdehyde contents and accelerated the superoxide anion production rate in dose-dependent manners, which might be associated with lower antioxidant enzyme activities, especially at higher concentrations of the herbicides. Acetochlor and fluoroglycofen inhibited grapevine growth in the growth season one-year after herbicide treatment, and stem height was inhibited by up to 55.4% and 88.0%, respectively. Taken together, these results suggest that both herbicides are detrimental for grape photosynthesis and this might be associated with increased oxidative stress in the first year, while growth inhibition in the second year might be due to after effects of herbicide treatment.

1. Introduction

Herbicides are widely used to control weeds in agriculture. However, in recent years, consumers have become increasingly aware of the impact of farming practices on the environment and food quality, especially any deleterious effects. Thus, herbicide toxicity against non-target crop species continues to warrant thorough investigation.

Various strains and cultivars within the same crop species show marked variability in tolerance to different herbicides and other environmental stresses such as drought and low temperature [1–6]. Various herbicides have different targets and modes of action, which results in variations in the performances of different plants [7,8]. Moreover, the old and young leaves from an individual plant can show differential resistances to herbicides and environmental stresses. Some reports have indicated that older leaves are more tolerant to herbicides and environmental stresses [9]; however, in some species, such as pea, cucumber and squash, younger leaves are often more tolerant to chemical and environmental stresses [10–13].

Many studies have demonstrated that herbicides can inhibit plant growth [5,14,15], reduce chlorophyll contents and photosynthesis [16–24]. Application of herbicides can also lead to decreases in the maximal photochemical efficiency of photosystem (PS) II (F_v/F_m), the photochemical quenching coefficient (Q_P) and the quantum yield of PSII electron transport (Φ_{PSII}) [14,16,19]. In addition, long-term exposure to herbicides may cause imbalances in reactive oxygen species that could ultimately damage the plant. Nevertheless, plants have evolved various protective strategies to minimize herbicide toxicity. One of these protective mechanisms is the antioxidant system that operates via the sequential and simultaneous actions of various enzymes, including superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT). The activities of some enzymes increase at low herbicide concentrations but decrease at higher exposures [25,26]. The effects of herbicides on enzyme activities may differ due to the length of exposure time and the time at which enzyme activity is measured.

Acetochlor (a chloroacetanilide) and fluoroglycofen (a diphenylether) can both be absorbed by grapevine roots. Diphenylether herbicides inhibit protoporphyrinogenoxidase, while acetochlor can disturb and inhibit the photosynthetic electron transport [24]. In China, both of these herbicides are used commonly on farmland largely due to their low cost. But in Qufu, Shandong,

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; F_v/F_m, efficiency of photosystem II; MDA, malondialdehyde; P_n, net photosynthetic rate; NPQ, non-photochemical quenching coefficient; POD, peroxidase; Q_P, photochemical quenching coefficient; PS, photosystem; Φ_{PSII}, quantum yield of PSII electron transport; SOD, superoxide dismutase; G_s, stomatal conductance.

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China, we observed in two of vineyards which was applied with paraquat, acetochlor and fluoroglycofen perennially, the grape leaves grown dark and round, however the photosynthesis rate decreased compared with the control, which used artificial weeding. In this present study, we studied the effects of acetochlor and fluoroglycofen on the photosynthesis and antioxidant enzyme activities in non-target grape leaves. Moreover, the growth of the grape plants was examined in the second year in order to understand further the mechanisms of any detrimental effects caused by these chemicals.

2. Materials and methods

2.1. Plant and growth conditions

Experiment was conducted in a greenhouse at the Shandong Agriculture University, China. One-year old grapevines (*Vitis vinifera* × *Vitis* labrusca cv. Kyoho) were grown in plastic pots (25 cm in diameter) containing garden earth, sand and matrix soil (2:1:1) in a greenhouse operating at photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}, relative humidity of 75–80% and a photoperiod of 14/10 h light/dark at 25 °C. When the shoots had ten leaves, acetochlor or fluoroglycofen were sprayed on the soil as follows: (1) T_1, 2,246 g ai ha^{-1} acetochlor (according to the area of pots, 0.014 g ai per pot); (2) T_2, 11,230 g ai ha^{-1} acetochlor (0.070 g ai per pot); (3) T_3, 22,460 g ai ha^{-1} acetochlor (0.140 g ai per pot); (4) T_4, 37.5 g ai ha^{-1} fluoroglycofen (0.00023 g ai per pot); (5) T_5, 187.5 g ai ha^{-1} fluoroglycofen (0.00115 g ai per pot); (6) T_6, 375 g ai ha^{-1} fluoroglycofen (0.00230 g ai per pot). Simultaneously, control (CK) soil was sprayed with water. The experiment was performed twice with three plants each treatment. Once grapes had been exposed to herbicide for 30 d, physiological indices were analyzed on the leaves at the upper-node (13–14), middle-node (8–9) and bottom-node (3–4) in grape seedlings. During the experiment, the plants were irrigated with equal volume of water (guarantee the amount of water not flowing out of the pot) once every two days.

2.2. Gas exchange measurements

Measurements of the net photosynthetic rate (P_n) and the stomatal conductance (G_s) were made on the upper-node (13–14), middle-node (8–9) and bottom-node (3–4) leaves of grape seedlings using an open system (Ciras-2, PP Systems, Hitchin, UK).

2.3. Analysis of chlorophyll fluorescence

Chlorophyll florescence was measured with a FMS-2 pulse modulated fluorometer (Hansatech, UK). The minimal fluorescence (F_o) was determined by a weak modulated light which was low enough not to induce any significant variable fluorecence. A 0.8 s saturating light of 8000 μmol m^{-2} s^{-1} was used on dark-adapted leaves to determine the maximal fluorescence (F_m). Then the leaf was illuminated by an actinic light of 500 μmol m^{-2} s^{-1}. When the leaf reached steady-state photosynthesis, the steady-state fluorescence (F_s) was recorded and a second 0.8s saturating light 8000 μmol m^{-2} $s^{-1}$of was given to determine the maximal fluorescence (F_m') in the light-adapted state. The actinic light was then turned off; the minimal fluorescence in the light-adapted state (F_o') was determined by the illumination of the 3 s far red light.

The following parameters were then calculated: (1) Q_P, the photochemical quenching coefficient, $Q_P = (F_m' - F_s)/(F_m' - F_o')$; (2) F_v/F_m, maximal photochemical efficiency of PSII; (3) Φ_{PSII}, quantum yield of PSII electron transport, $\Phi_{PSII} = (F_m' - F_s)/F_m'$, [27]; (4) NPQ, non-photochemical quenching of chlorophyll fluorescence. $NPQ = (F_m - F_m')/F_m'$.

2.4. Measurements of pigment content

The chlorophyll and carotenoid content was determined spectrophotometrically in 80% acetone with a double beam spectrophotometer Unicam UV 550 (ThermoSpectronic, Cambridge, UK) according to Lichtenthaler [28].

2.5. Measurements of lipid peroxidation and superoxide radical

The comparative rates of lipid peroxidation were assayed by determining the levels of malondialdehyde (MDA) in 0.5 g aliquots of leaves. Malondialdehyde is a product of lipid peroxidation and was assayed by the thiobarbituric acid (TBA) reaction [29].

The production rate of O_2^- was determined according to Elstner and Heupel [30] by monitoring the nitrite formation from hydroxylamine in the presence of O_2^-.

2.6. Assays of antioxidant enzyme activities

After herbicide treatment for 30 d, the different node leaves were harvested and immediately frozen in liquid N_2, and then stored at −80 °C until experimental analyses. Frozen leaves (0.5 g) were crushed into fine powder in a mortar and pestle under liquid N_2. Cell-free homogenates for antioxidant enzyme assays were prepared essentially by the method described by Jiang and Zhang [31]. Soluble proteins were extracted by homogenizing the powder in 10 ml of 50 mM natrium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone (PVP), with addition of 1 mM ascorbate in the case of ascorbate peroxidase (APX) assay. The homogenate was centrifuged at 15,000*g* for 30 min at 4 °C and the supernatant was used for the following enzyme assays. Total superoxide dismutase (SOD) activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Giannopolitis and Ries [32]. Catalase (CAT) activity was determined by following the consumption of H_2O_2 (extinction coefficient 39.4 mM^{-1} cm^{-1}) at 240 nm for 3 min [33]. Ascorbate peroxidase (APX) activity was measured by monitoring the decrease in absorbance at 290 nm (extinction coefficient 2.8 mM^{-1} cm^{-1}) [34]. Guaiacol peroxidase (POD) activity was assayed following the method described by Cakmak and Marschner [35]. Protein content was determined according to the method of Bradford [36].

2.7. Growth analysis in 2011

The treated grape seedlings were germinated in 2011, when the shoots of control had eight leaves, the grape seedlings treated with acetochlor and fluoroglycofen were photographed, respectively; the length of stems were measured, the leaves and roots of each plantlet were weighed (roots were first rinsed with distilled water) to calculate the ratio of root to shoot (FW).

2.8. Statistical analysis

Each reported data are the mean ± standard error (SE) of six replicates combined in the two experimental repeats. Statistical analyses were performed by analysis of variance (ANOVA) using SPSS version 13.0 (SPSS, Chicago, USA) and comparisons between the mean values were made by least significant difference (LSD) at a 0.05 probability level.