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EFFECTS OF HERBICIDE DRIFT ON CHLOROPHYLL FLUORESCENCE AND ANTIOXIDANT ENZYME LEVELS OF VARIOUS TYPES OF FRUIT TREES

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Abstract

We studied the effects of glyphosate and glufosinate-ammonium (GLA) herbicides on the photosynthetic and antioxidant enzyme capacities in the leaves of various non-target fruit species that are sometimes subjected to herbicide drift. Glyphosate is a slow-acting systemic herbicide and GLA a new, nonselective contact (non-conducting) herbicide. Glyphosate and GLA were sprayed (at the recommended doses) onto 1-year-old saplings of 'Fuji' apple (*Malus pumila* Mill.), 'Chunmi' peach (*Prunus persica* [L.] Batsch), and 'Summer Black' grape (*Vitis vinifera* L. × *Vitis labrusca* L.) growing in the Zhengzhou Fruit Research Institute of the Chinese Academy of Agricultural Sciences. Glyphosate and GLA triggered distinct injuries at different times, and greatly reduced the Soil-Plant Analyses Development (SPAD) readings of apple, peach, and grape leaves. Both herbicides reduced photosystem II efficiencies under light conditions, increased non-photochemical quenching (NPQ), and increased the levels of malondialdehyde and antioxidant enzyme levels. The chlorophyll fluorescence transients differed greatly between sprayed fruit trees and control. GLA reduced the performance indices (the PI_{ABS} values) of apple, peach, and grape leaves by 14.89%, 15.53%, and 18.05%, respectively; the glyphosate-induced reductions were 68.33%, 30.41%, and 8.20%. Thus, photosystem II activity was reduced by glyphosate and GLA application in all three trees, associated with reduced antioxidant enzyme levels. All species were less susceptible to glyphosate than GLA and the apple was less susceptible than the peach and grape. However, no tree recovered from glyphosate-induced injury, whereas apple and peach (but not grape) trees recovered from GLA-induced injury.

Key words: *N*-phosphonomethyl-glycine, Glufosinate-ammonium (GLA), Spray drift, Chlorophyll a fluorescence, Antioxidant enzyme, Fruit tree.

Abbreviations: CAT, catalase; GLA, glufosinate-ammonium; MDA, malondialdehyde; POD, guaiacol peroxidase; PS, photosystem; PSII, photosystem II; SOD, superoxide dismutase.

Introduction

Herbicides have become an important technical measure for weed control in orchards. The principal herbicide applied in Chinese fruit plantations is glyphosate, a slow-acting systemic herbicide which is extremely "safe" for animals, that's why the herbicide is so popular (Pieniazek *et al.*, 2003). Glyphosate could kill plants, because it inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), reduces the content of tryptophan, tyrosine and phenylalanine required for the synthesis of protein and impacts the synthesis of lignin, flavonoids and other compounds required by cell walls, to mount defenses against pathogens and in many other processes(Duke & Powles, 2008). Plant chlorophyll synthesis is blocked by the inhibition of cell division. Accumulation of free ammonia and the associated decline in photosynthetic capacity disturb protein synthesis and trigger metabolic dysfunction so that plant growth slows or ceases (Gougler & Geiger, 1981; Webster, 1977 and Carvalho *et al.*, 2016). Over 50 commercial preparations containing glyphosate are available; all are much more toxic than glyphosate alone because of the presence of surfactants and carrier compounds (Kwiatkowska *et al.*, 2013). Commercial glyphosate-based concentrates contain ≥41% (w/v) glyphosate, but the level thereof in formulations for domestic use may be only 1% (w/v) (Bradberry *et al.*, 2004). Glufosinate-ammonium (GLA) is an organophosphorus herbicide, as is glyphosate. However, they work in different ways; the former herbicide is a new, nonselective, contact (non-conducting) herbicide. Glyphosate kills roots, whereas GLA kills leaves by inhibiting glutamine synthase (GS), dysregulating nitrogen metabolism, and triggering ammonium ion accumulation that destroys the cell membrane and blocks photosynthesis, ultimately causing death (Hoerlein, 1994; Calas *et al.*, 2016). In recent years, people have paid more attention on the impacts (particularly those that are negative) of agricultural practice for atmospheric environment, soil environment and fruit quality. Thus, herbicide toxicity toward non-target crops warrants thorough investigation (Tan *et al.*, 2012). Crops differ markedly in terms of herbicide tolerance and resistance to environmental stressors (Smart, 1994; Choi *et al.*, 1999). Herbicides differ in terms of their targets and modes of action, thus affecting non-target plants to different extents (Kaushik, 2010; Sunohara *et al.*, 2010).

Many studies have shown that herbicides inhibit plant growth, reduce chlorophyll levels and photosynthesis (Saladin *et al.*, 2003; Kopsell *et al.*, 2011). Herbicides reduce the quantum yield of PSII-mediated electron transport (ΦPSII), maximal PSII photochemical quantum efficiency (F_v/F_m) and photochemical quenching coefficient (Q_P) (Bigot *et al.*, 2007; Macedo *et al.*, 2008). Plants can minimize the toxicity of herbicides through an antioxidant system, which including peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD). Some enzymes activities decrease at high concentrations of herbicides but rise at low concentrations (Štajner *et al.*, 2003; Jiang & Yang, 2009). Chlorophyll fluorescence is an effective non-invasive measure of the status of PSII in photosynthesis (Munir *et al.*, 2015; Neuner & Larcher,

1990; Fryer *et al.*, 1995). When illuminated continuously, leaves adapted to darkness exhibit characteristic changes of chlorophyll fluorescence intensity. The Kautsky transient rises rapidly and then declines more slowly toward a stable state. The rising phase of transients afforded many accurate information reflects the main reactions of photosynthesis (Krause & Weis, 1991). Because the fast fluorescence is sensitive to environment, the JIP test is a useful tool to convert the original fluorescence values into physiological expressions of quantifying PSII when exploring photosynthetic status *in vivo* (Strasserf & Srivastava, 1994; Krüger *et al.*, 1997). We used this approach (which has not previously been applied to evaluate fruit trees) to explore the response of the PSII system to herbicide stress.

Glyphosate (a systemic herbicide) and GLA (a nonselective, contact non-conducting herbicide) inhibit EPSPS and GS, respectively; both herbicides can inhibit photosynthetic electron transport. Glyphosate and GLA are generally applied to farmlands because they are cheap. However, accidents are common, compromising herbicide efficacy and also the yield and quality of crops growing near weedy fields. Climate (particularly wind and temperature) affects herbicide drift. Some herbicides are more volatile than others, and may drift with wind. Inappropriate spraying can disperse many herbicide droplets into the air, again triggering drift. As agriculture and forestry practices became widely mechanized, many farmers began to ignore the recommended precautions, change the herbicide levels and spray indeed spraying non-target trees. It is very important to explore whether herbicide drift might damage fruit trees and how to mitigate this problem (Al-Khatib *et al.*, 1993; De Snoo & Van der Poll, 1999). Here, to research the influences both of glyphosate and GLA herbicides on photosynthetic and antioxidant enzyme capacities of leaves of non-target fruit trees, we measured chlorophyll a fluorescence and antioxidant enzyme levels.

Materials and Methods

Experimental design: The study was completed in the Zhengzhou Fruit Research Institute of the Chinese Academy of Agricultural Sciences. Potted plant materials were used (35 cm in diameter and 30 cm deep). Plastic pots contain garden soil, sand and matrix soil (2:1:1 v/v/v). Each pot contained the same amount of soil. When apple, peach, and grape shoots were at the 10-leaf stage, we sprayed 41% (w/v) glyphosate-isopropylammonium and herbicides onto the leaves at doses of 4.97 $g \cdot L^{-1}$ and 1.74 $g \cdot L^{-1}$, respectively; these are the doses recommended for control of several weed species; each tree was sprayed with 15 mL of solution. All seedlings had earlier exhibited good consistent growth. Simultaneously, the control leaves were sprayed with equal volumes of distilled water. We ensured that no liquid entered the soil. The experiment featured three plants per treatment (10 replicates), with each plant growing in an individual pot (Carvalho *et al.*, 2016). Throughout the experiment, all plants were watered (with the same volume of water) every 2 days; we ensured that no water flowed out of the pots.

Experimental material: We used 1-year-old saplings of 'Fuji' apple (*Malus pumila* Mill.), 'Chunmi' peach (*Prunus persica* [L.] Batsch), and 'Summer Black' grape (*Vitis vinifera* L. × *Vitis labrusca* L.) supplied by the Zhengzhou Fruit Research Institute of the Chinese Academy of Agricultural Sciences. Seedlings from these saplings were grown for 3 months and then used in experiments.

A glyphosate-based herbicide containing 41% (w/v) of an isopropylamine salt of *N*-(phosphonomethyl)-glycine (30% [w/v] glyphosate equivalent [ae]; Roundup®; Shaanxi Sunger Road Bioscience Co. Ltd., China), and an aqueous solution (200 g/L) of GLA, were used.

Assay of chlorophyll content: Chlorophyll contents were assayed in leaves that had been subjected to chlorophyll fluorescence measurements using a portable device (SPAD-502) (10 measurements/leaf) (Liu *et al.*, 2015). SPAD data were applied to estimate leaf photosynthetic capacity, because SPAD readings correlate significantly with chlorophyll content (Kumagai *et al.*, 2009).

Chlorophyll fluorescence: The portable pulse-amplitude-modulated (PAM) chlorophyll fluorometer (PAM-2500, Walz, Germany) connected to the computer via a data acquisition system (Pamwin-3) was used to measure the chlorophyll fluorescence parameters of fruit tree leaves. Each leaf was dark adapted for 30 min before measurement, and six measurements were made on each leaf (Guo *et al.*, 2006). The minimal fluorescence (F_o) and maximal fluorescence (F_m) were determined by turning on the measuring light (< 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and the saturated pulse light (> 8,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$), separately (Maxwell & Johnson, 2000; Guo *et al.*, 2005). Afterwards, the maximal fluorescence yield in the light-adapted state (F_m') were determined by turning on the active light (538 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and the saturated pulse light (> 8,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$). When steady-state photosynthesis was attained, the fluorescence (F_s) was recorded, and then the minimal fluorescence yield of the light-adapted state (F_o') was determined by turning on the far-red light (Van Kooten, 1990; Kanwal *et al.*, 2011). The F_v/F_m, Q_P, ΦPSII and the extent of non-photochemical quenching of chlorophyll fluorescence (NPQ) were calculated according to the formula of Genty *et al.* (1989) in Table 1.

JIP evaluation of the O-J-I-P chlorophyll fluorescence transients: Rapid induced kinetics curve was determined using the Wald instrument described above. Leaves were dark adaption for 30 min before each measurement, and the quick diversification of fluorescence signals recorded from 10 μs to 300 ms thereafter were reflected on O-J-I-P curve after instantaneous exposure to red light of 650 nm (3,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Tan *et al.*, 2011). These transients were subjected to JIP testing using the following data: fluorescence intensity at 20 μs (F_O); maximal fluorescence intensity (F_M); fluorescence intensity at 300 μs (the K-step) ($F_{300\mu s}$); fluorescence intensity at 2 ms (the J-step) (F_J); and fluorescence intensity at 30 ms (the I-step) (F_I). The formulae of Table 2 show how to obtain abovementioned biophysical parameters that quantify PSII energy flow from the original data (Stirbet & Govindjee, 2011; Ceppi *et al.*, 2012).

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Antioxidant enzyme levels and malondialdehyde content: After herbicide treatment, leaves were harvested. The harvested leaves were immediately brought back to the laboratory in an ice box, and stored in a refrigerator at -80°C after quick frozen in liquid nitrogen. The stored leaf (0.2g) put into a centrifugal tube was crushed into powder with a Scientz-48 High-throughput Tissue Grinder under liquid nitrogen. Then the power was mixed with 1.8 ml pre-cooled grinding fluid [0.05 $mol \cdot L^{-1}$ potassium phosphate buffer (pH 7.8) containing 1 $mmol \cdot L^{-1}$ EDTA and 1% (w/v) polyvinylpyrrolidone (PVP)]. Each homogenate was centrifuged at 15,000 $r \cdot min^{-1}$ for 20 min at 4°C and the supernatant was assayed in terms of enzyme and malonaldehyde (MDA) levels (Tan *et al.*, 2012). The SOD activity was assayed by riboflavin-nitroblue tetrazolium (NBT) method (Dhindsa *et al.*, 1981). The POD activity was determined by guaiacol-colorimetric method (Jun *et al.*, 2017). The CAT activity was measured by ammonium molybdate method using ultraviolet spectrophotometer (Nakano & Asada, 1981). MDA content was assayed by the thiobarbituric acid method of Zhang & Kirkham (1994).

Table 1. Chlorophyll fluorescence parameters.

Fluorescence intensity indicator	Illustration/formula
F_s: steady-state fluorescence	The steady-state fluorescence level immediately prior to the flash
F_o: minimal fluorescence (dark)	Fluorescence intensity with all PSII reaction centers open while the photosynthetic membrane is in the non-energized state
F_m: maximal fluorescence (dark)	Fluorescence intensity with all PSII reaction centers closed. This is the classical maximum fluorescence level in the dark- adapted state
F_o': minimal fluorescence (light)	Fluorescence intensity with all PSII reaction centers open in any light-adapted state
F_m': maximal fluorescence (light)	Fluorescence intensity with all PSII reaction centers closed in any light-adapted state
F_v: variable fluorescence (dark)	Maximum variable fluorescence in the state at which all non-photochemical processes are at their minima ($F_m–F_o$)
F_v': variable fluorescence (light)	Maximum variable fluorescence in any light-adapted state. ($F_m'–F_o'$)
Q_P: photochemical quenching coefficient	$(F_m'–F_s)/(F_m'–F_o')$
NPQ: non-photochemical quenching of chlorophyll fluorescence	$(F_m –F_m')/F_m'$
F_v/F_m: maximal photochemical quantum efficiency of PSII	$(F_m–F_o)/F_m$
ΦPSII: quantum yield of PSII electron transport	$(F_m'–F_s)/F_m'$

Table 2. Formulae and terms used to create dynamic O-J-I-P fluorescence induction curves.

Fluorescence parameter	Explanation	Formula
F_O	Fluorescence intensity at 20 μs	$F_O=F_{20\ \mu s}$
F_J	Fluorescence intensity at the J-step (2 ms)	
F_I	Fluorescence intensity at the I-step (30 ms)	
F_M	Maximal fluorescence intensity	$F_M= F_P$
F_V	Relative variable fluorescence intensity	$F_V=F_M–F_O$
V_J	Relative variable fluorescence intensity at the J-step	$V_J=(F_{2ms}–F_O)/(F_M–F_O)$
M_O	Approximate initial slope of the fluorescence transient	$M_O=4\cdot(F_{300\mu S}–F_O)/F_V$
Φ_{Po}	Maximum quantum yield; primary photochemistry (at t=0)	$\Phi_{Po}=TR_O/ABS= [1–(F_O/F_M)] =F_V/F_M$
Ψ_O	Probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (at t=0)	$\Psi_O =ET_O/TR_O=1–V_J$
Φ_{Eo}	Quantum yield for electron transport (at t=0)	$\Phi_{Eo}=ET_O/ABS=[1–(F_O/F_M)]\cdot\Psi_O$
PI_{ABS}	Performance index on an absorption basis	$PI_{ABS}=(RC/ABS)\cdot[\Phi_{Po}/(1–\Phi_{Po})]\cdot[\Psi_O/(1–\Psi_O)]$
ABS/RC	Absorbed energy flux per reaction center (RC) (at t=0)	$ABS/RC=M_O\cdot(1/V_J)\cdot(1/\Phi_{Po})$
TR_O/RC	Trapped energy flux per RC (at t=0)	$TR_O/RC= M_O\cdot(1/V_J)$
ET_O/RC	Electron transport flux per RC (at t=0)	$ET_O/RC= M_O\cdot(1/V_J)\cdot\Psi_O$
DI_O/RC	Dissipated energy flux per RC (at t=0)	$DI_O/RC= (ABS/RC)–TR_O/RC$
ABS/CS	Absorbed energy flux per cross-section (CS)	$ABS/CS=ABS/CS_{Chl}$=Chl/CS or $ABS/CS_O=F_O$ or $ABS/CS_M=F_M$
TR_O/CS	Trapped energy flux per CS	$TR_O/CS =\Phi_{Po}\cdot(ABS/CS)$
ET_O/CS	Electron transport flux per CS	$ET_O/CS =\Phi_{Po}\cdot\Psi_O\cdot(ABS/CS)$
DI_O/CS	Dissipated energy flux per CS	$DI_O/CS = (ABS/CS)–(TR_O/CS)$
RC/CS	The proportion of active PSII reaction centers per excited CS	$RC/CS =\Phi_{Po}\cdot(V_J/M_O)\cdot ABS/CS$

Note: tF_M=time to reach F_M (ms). When $t=tF_M$, CSo becomes CS_M, $ABS/CS_M\approx F_M$

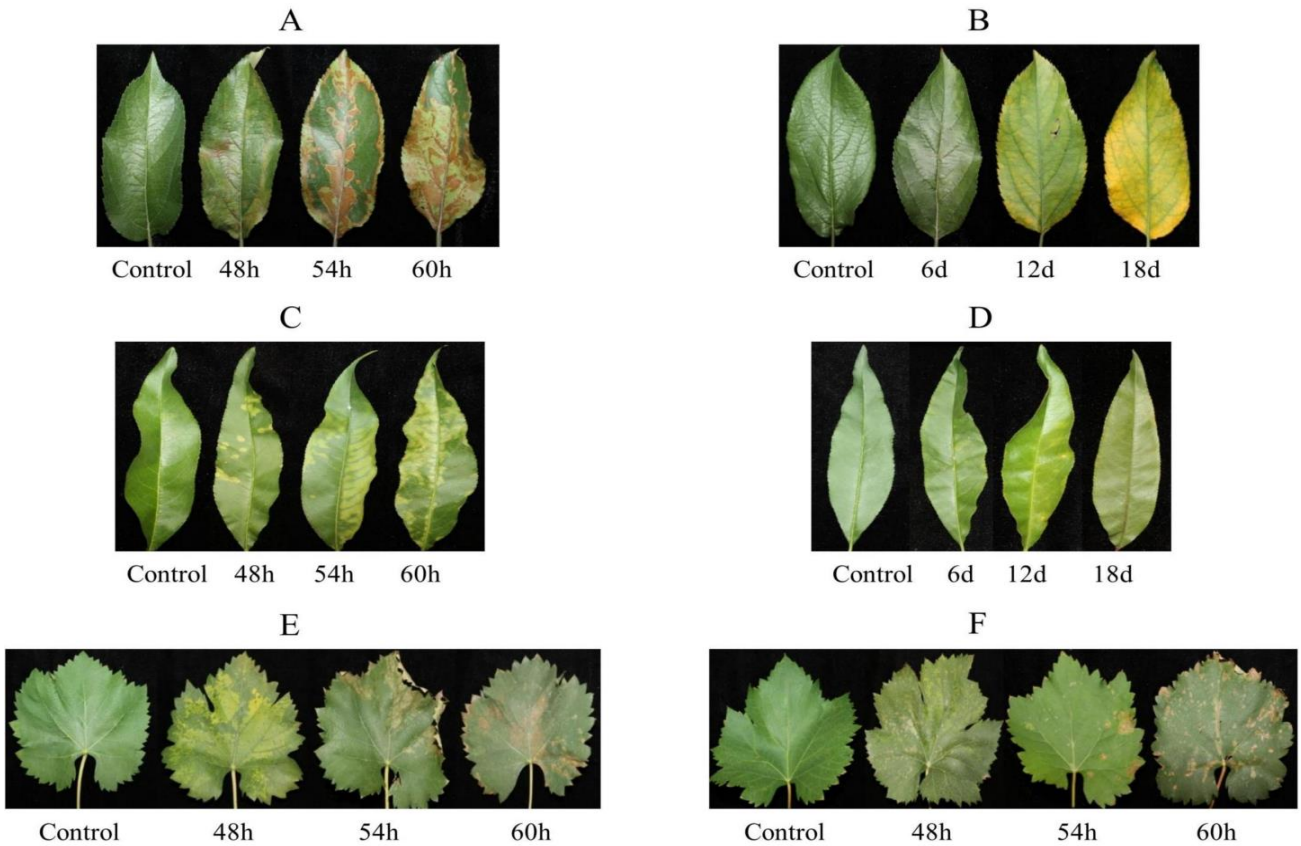

Fig. 1. Damage to apple, peach, and grape leaves evident at different times after spraying with glyphosate and GLA. A: GLA, apple; B: glyphosate, apple; C: GLA, peach; D: glyphosate, peach; E: GLA, grape; F: glyphosate, grape.

Statistical analysis: The significance of difference between treatments was evaluated via one-way analysis of variance (ANOVA) by SPSS ver. 18.0. The least significant differences (LSDs) between treatments were compared; a probability of 0.05 was taken to reflect significance. Enzyme activities were calculated and plotted with the aid of SigmaPlot ver. 10.0. Contour maps were prepared using Origin ver. 9.0.

Results

Effects of herbicides on leaves: Glyphosate and GLA triggered different injury symptoms on leaves of the three fruits at different times. The leaves of apple browned by 48h, dried by 54h, and fell by 60h after GLA treatment (Fig. 1A); glyphosate triggered slow yellowing by 12 d, and serious yellowing but not drying by 18d (Fig. 1B). Peach leaves began to show chlorotic spots 48 h after GLA treatment; the spots expanded to form slices by 60 h (Fig. 1C). Glyphosate caused slow yellowing to 12 d, and mature leaves became stiff and began to fall by 18 d (Fig. 1D). Both GLA and glyphosate caused browning of grape leaves by 48 h, and serious yellowing, then falling by 60 h (Figs. 1E, F). Furthermore, the changes were not reversible in trees treated with glyphosate, but apple and peach tips grew new leaves after GLA treatment.

The effects of herbicides on the relative chlorophyll contents (SPAD readings) of different tree species: The relative chlorophyll contents changed significantly among various fruit trees (Fig. 2). Under GLA stress, the leaves of the three trees all fell on the third day (August 8, 2017) after treatment. The falls in SPAD readings were 6.30% for apple, 12.95% for peach, and 19.53% for grape trees over the 3 days of GLA stress, compared to the levels in controls. Peach and grape trees exhibited more marked changes than apple trees. Under glyphosate stress, the leaves of the three trees fell at different times after spraying. Compared to the controls, glyphosate reduced the SPAD readings of apple, peach, and grape leaves by 80.77% (August 24, 2017), 16.20% (August 24, 2017), and 11.73% (August 8, 2017), respectively.

Effects of herbicides on chlorophyll fluorescence parameters in different tree species: Glyphosate significantly reduced the F_v/F_m ratio of grape leaves by 29.98%, but not the ratios of apple and peach leaves in figure 3. Compared to the control, GLA reduced the F_v/F_m ratios of apple, peach, and grape leaves by 55.78%, 57.60%, and 73.27%, respectively (Fig. 3A). Glyphosate significantly reduced the ΦPSII of peach leaves by 39.05%, but not the values of apple and grape leaves. GLA significantly reduced the ΦPSII values of peach and grape leaves by 76.03% and 88.48%, but not that of apple leaves (Fig. 3B). Neither glyphosate nor GLA significantly affected the Q_P values of apple, peach, or grape leaves (Fig. 3C). The NPQ of glyphosate-treated peach leaves was much higher (35.46%) than the control value, but apple and grape trees were not affected. The NPQ values of GLA-treated apple, peach, and grape leaves all decreased significantly compared with the control (77.67%, 72.28%, and 73.83%, respectively) (Fig. 3D).

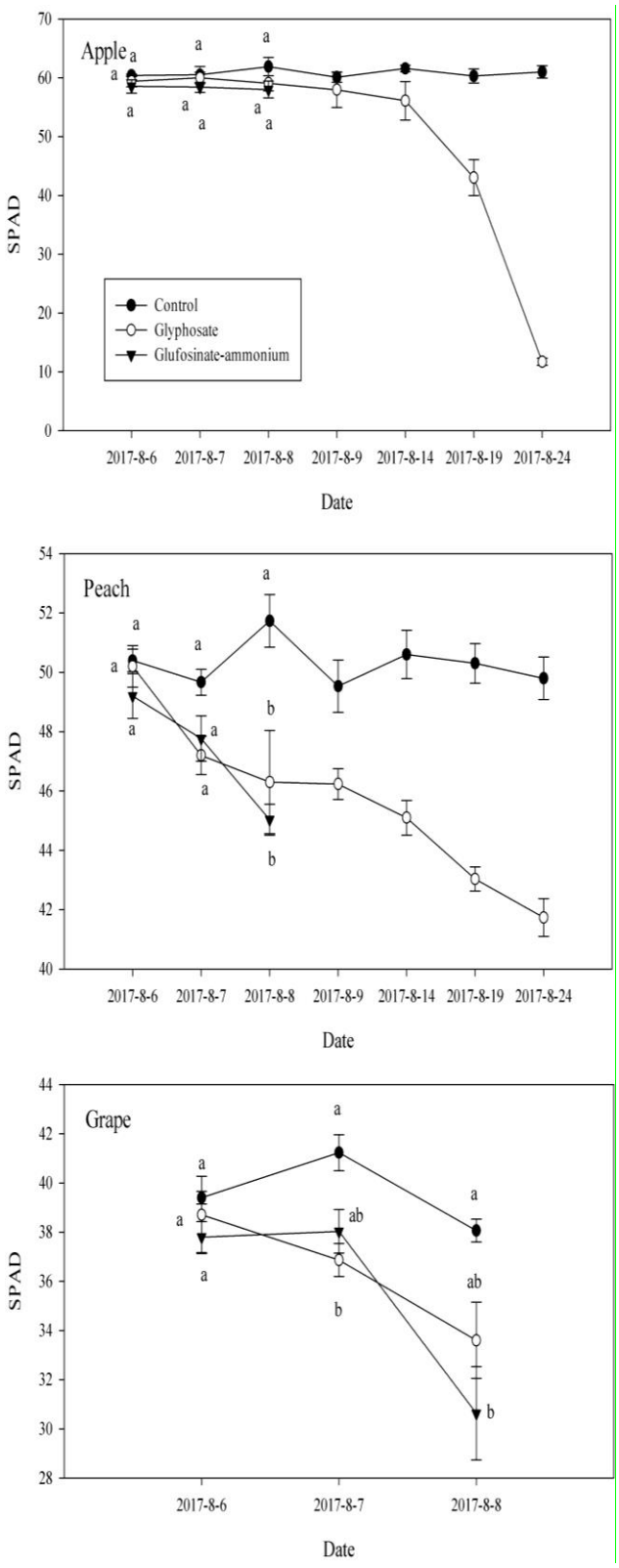

Fig. 2. Effect of glyphosate and GLA on the relative chlorophyll contents of apple, peach, and grape. Lines marked with different lower-case letters indicate significant differences between treatments ($p<0.05$).

Effects of herbicides on chlorophyll fluorescence kinetics curve of different tree species: Figure 4 shows the fast chlorophyll fluorescence kinetics of grape leaves at 48 h and 3 d, and apple and peach leaves at 3 and 18 d, after exposure to herbicides. The baseline shapes of all treatments were similar. However, after herbicide treatment, the O-J-I-P curve differed greatly between glyphosate- and GLA-treated plants and controls. As is true of other green plants, the chlorophyll *a* fluorescence curves commenced at initial F_O intensities to peak at P or F_M values when fronds were exposed to saturating actinic light. The F_J and F_I were sequentially present to between F_O and F_M. The JIP values decreased at different times after treatment. The overall slopes of the curves for peach and grape trees fell at both 18 d and 3 d, but the slope of apple trees in different periods had no significant difference compared with the control.

Effects of herbicides on chlorophyll fluorescence kinetics (O-J-I-Ps) parameters: The fast fluorescence kinetics on the third day revealed that variations in the trends of all three fruit trees were similar under glyphosate and GLA stress conditions (Fig. 5). GLA stress reduced the performance indices (the PI_{ABS} values) by 14.89%, 15.53%, and 18.05% in apple, peach, and grape trees; the glyphosate-induced reductions were 68.33%, 30.41%, and 8.20%. The approximate initial slopes of the fluorescence transients (M_O values) increased by 66.38%, 39.97%, and 64.42% when apple, peach, and grape trees were subjected to GLA stress; and by 5.46%, 25.24%, and 87.08% when glyphosate stress was imposed. The ABS/RC ratios increased by 21.78%, 41.47%, and 31.82% in apple, peach, and grape trees under GLA stress, and by 47.63% when apple trees were subjected to glyphosate stress. The ABS/CSo ratios increased by 17.24%, 42.08%, and 10.04% when apple, peach, and grape trees were under GLA stress; and by 13.68% in grape trees under glyphosate stress. However, the quantum efficiencies (also termed flux ratios or yields; ΦPo, ΦEo, Ψ_O) and other parameters were minimally decreased by either glyphosate- or GLA-induced stress.

Effects of herbicides on antioxidant enzyme activities and MDA levels in the leaves of different tree species: Herbicide treatments significantly changed antioxidant enzymes activities in the leaves of three tree species on the third day. GLA significantly reduced the SOD activities of apple, peach, and grape leaves (compared to controls). Glyphosate significantly reduced the SOD activity of grape leaves, but not apple or peach leaves (Fig. 6-1A). The CAT activities in apple, peach and grape leaves were significantly lower than those of controls after GLA treatment, but significantly lower in grape leaves only after glyphosate treatment. Glyphosate had no significant effect on CAT activities of apple or peach leaves (Fig. 6-1B). Glyphosate significantly reduced the POD activity of grape leaves, but not those of apple or peach leaves. Compared to controls, GLA significantly reduced the POD activities of apple, peach, and grape leaves (Fig. 6-1C). In apple, peach, and grape leaves, MDA levels increased (compared to control levels) by 47.46%, 96.50%, and 44.90% after GLA treatment and by 19.99%, 34.23%, and 34.13% after glyphosate treatment, respectively (Fig. 6-1D).

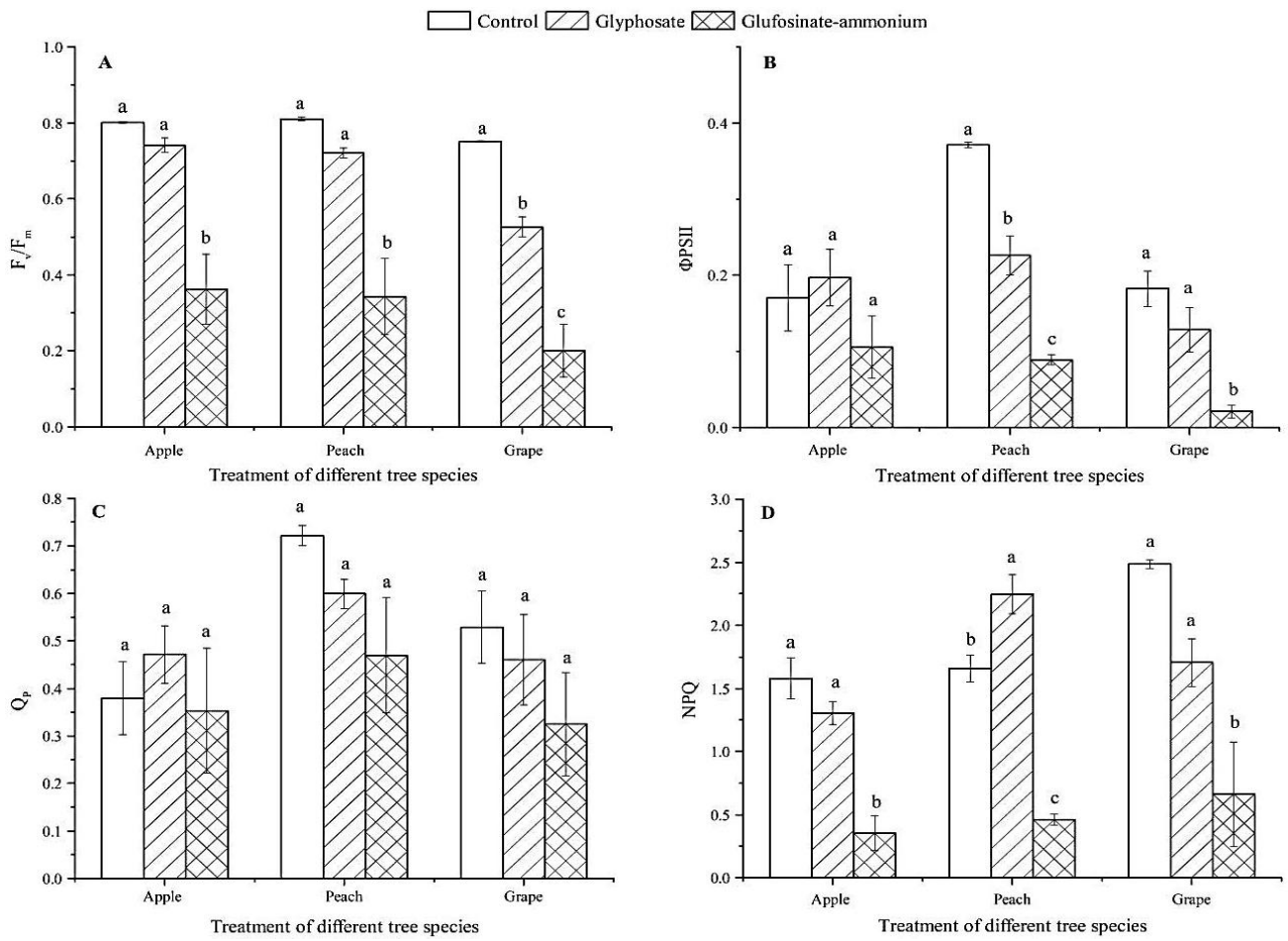

Fig. 3. Effect of herbicides on chlorophyll fluorescence parameters in the dark-adapted states of different tree species at 72 h (on August 8, 2017) after treatment. A: F_v/F_m, the maximal PSII photochemical quantum efficiency; B: ΦPSII, quantum yield of PSII electron transport; C: Q_P, photochemical quenching coefficient; D: NPQ, non-photochemical quenching of chlorophyll fluorescence. Columns marked with different lower-case letters indicate significant differences between treatments ($p<0.05$).

Discussion

As agriculture has become increasingly mechanized, chemical spraying has become more random; non-target crops are now not protected. In addition, some farmers spray high levels of chemicals to optimize killing. Valuable trees may thus be affected by herbicide drift. Many herbicides inhibit PSII (Oettmeier, 1999): atrazine prevents electron transfer to the proton quinone pool though binding the D1 protein of PSII (Rutherford & Krieger-Liszkay, 2001); clomazone depresses electron transport in chloroplasts thylakoid membrane (Kaňa *et al.*, 2004); acetochlor reduces the photochemical efficiency of PSII in terms of both light-induced and NPQ (Tan *et al.*, 2012); flumioxazin inhibits protoporphyrin oxidase, reducing the net photosynthetic rate and GS levels of grape leaves (Bigot *et al.*, 2007). We studied both glyphosate and GLA because their modes of action differ. Glyphosate kills roots by inhibiting EPSPS (Duke & Powles, 2008); GLA kills by inhibiting GS. Both herbicides inhibit photosynthetic electron transport. We explored herbicide drift damage to fruit trees, and how to mitigate the associated phytotoxicity.

The herbicides blocked plant chlorophyll synthesis. All three plants yellowed, browned and then died after being sprayed with glyphosate and GLA. The symptoms changed over time (Fig. 1); the SPAD readings of all test plants fell (Fig. 2). Furthermore, glyphosate-induced injuries were not reversible, but apple and peach tips grew new leaves after exposure to GLA. Liu *et al.*, (2015) reported that total chlorophyll content decreased during the first 3 years of acid stress but then increased in the next year.

Chlorophyll fluorescence is practically only related to PSII, the function of which is controlled by many environmental parameters; chlorophyll fluorescence yields a large number of information about stress conditions. Glyphosate and GLA reduced the F_v/F_m and ΦPSII levels of apple, peach, and grape leaves, and glyphosate significantly increased the peach NPQ (Fig. 3), conforms to the consequences of Frankart *et al.*, (2003); norflurazon and paraquat (each at 100 $\mu g \cdot L^{-1}$) observably decreased the F_v/F_m ratio, the ΦPSII, and the Q_P, but significantly increased the NPQ (Frankart *et al.*, 2003). Flumioxazin and terbutryn, respectively, reduced the NPQs of grape and *Vicia faba* (Bigot *et al.*, 2007; Piñol & Simón, 2009). We found that the NPQs of apple, peach, and grape leaves exposed to GLA decreased significantly compared with the control values (Fig. 3D). The NPQ protects blades from light-induced injury, and has a linear relationship with the energy dissipation of the leaves (Maxwell & Johnson, 2000). Tan (2012) found that both acetochlor and fluoroglycofen increased the NPQ and decreased the ΦPSII, consistent with our results. Both of glyphosate and GLA triggered non-radiative energy dissipation and decreased the activity of PSII.

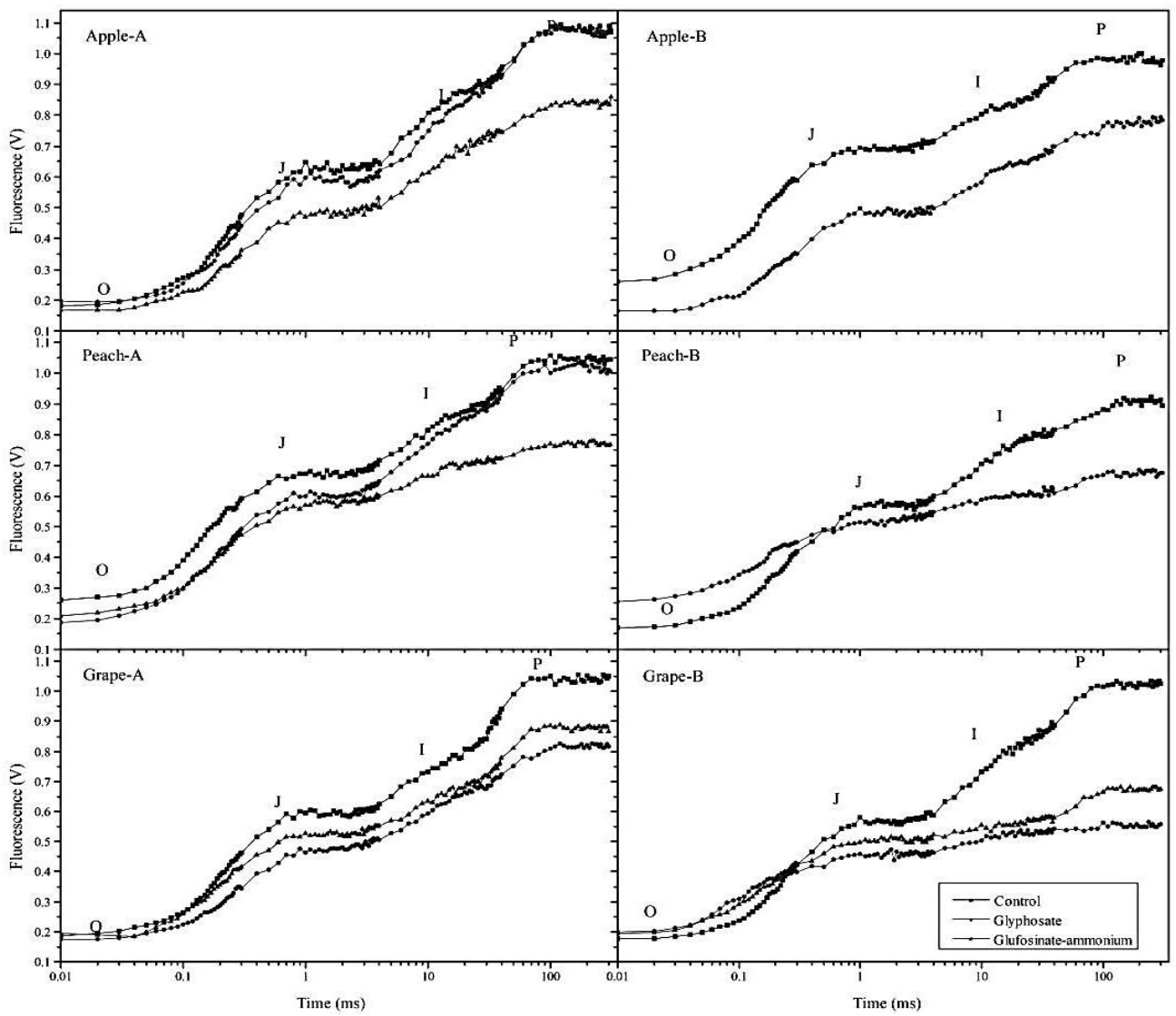

Fig. 4. Changes in fluorescence kinetics (O-J-I-Ps), plotted on a logarithmic time scale from 10 μs to 300 ms, of dark-adapted leaves of various tree species after herbicide treatment. Apple-A: the fluorescence kinetic curves of apple 3 d after treatment; Apple-B: the fluorescence kinetic curves of apple 19 d after treatment; Peach-A: the fluorescence kinetic curves of peach 3 d after treatment; Peach-B: the fluorescence kinetic curves of peach 19 d after treatment; Grape-A: the fluorescence kinetic curves of grape 48 h after treatment; Grape-B: the fluorescence kinetic curve of grape 3 d after treatment.

Plants exhibit direct or indirect oxidative damage caused by herbicides and express a suite of antioxidant enzymes and antioxidants per se, but the induction of enzyme activities is often accompanied by a disturbance in redox homeostasis (a signal of oxidative stress; Platiša *et al.*, 2008 and Qian *et al.*, 2008). MDA is the end-product of membrane lipid peroxidation and accumulates when reactive oxygen species engage in toxic activities. The responses of antioxidant enzymes to herbicides which trigger the accumulation of active oxygen vary by treatment and assay times; concentration- and time-dependent effects are also in play (Hassan & Alla, 2005; Geoffroy *et al.*, 2004 and Yoon *et al.*, 2011). We discovered that both glyphosate and GLA exacerbated membrane lipid peroxidation, thus reducing the levels of antioxidant enzymes. In agreement with other reports, we found that both glyphosate and GLA reduced CAT, POD, and SOD activities and increased MDA contents in all three fruit leaves (Fig. 6-1). As time progressed, the CAT, POD, and SOD activities fell significantly in apple leaves, but MDA contents increased significantly compared with controls group in glyphosate-treated plants on the 19th day.

Chlorophyll *a* fluorescence has been extensively used to monitor the activity of PSII and the processes affecting such activity (Mehta *et al.*, 2010). The shapes of the O-J-I-P fluorescence transients were similar in all three plants before treatment, but differed greatly between glyphosate- and GLA-treated plants and controls. The JIP values fell in treated plants, at different times. However, the herbicides reduced the slopes of the overall peach and grape curves at both 18 d and 3 d, but did not affect the apple curve. Strasser (2000) derived a multiparametric combination of the three-step of photosynthesis, thus establishing the PI_{ABS} reflecting photosynthetic capacity (Hermans *et al.*, 2003). The herbicides reduced the PI_{ABS} values by 14.89%, 15.53%, and 18.05% in apple, peach, and grape trees sprayed with GLA and by 68.33%, 30.41%, and 8.20% in trees sprayed with glyphosate. The PI_{ABS} values were significantly lower than those of controls, was consistent with the conclusion of Tan (2011), who discovered that the PI_{ABS} fell suddenly as the temperature rose.

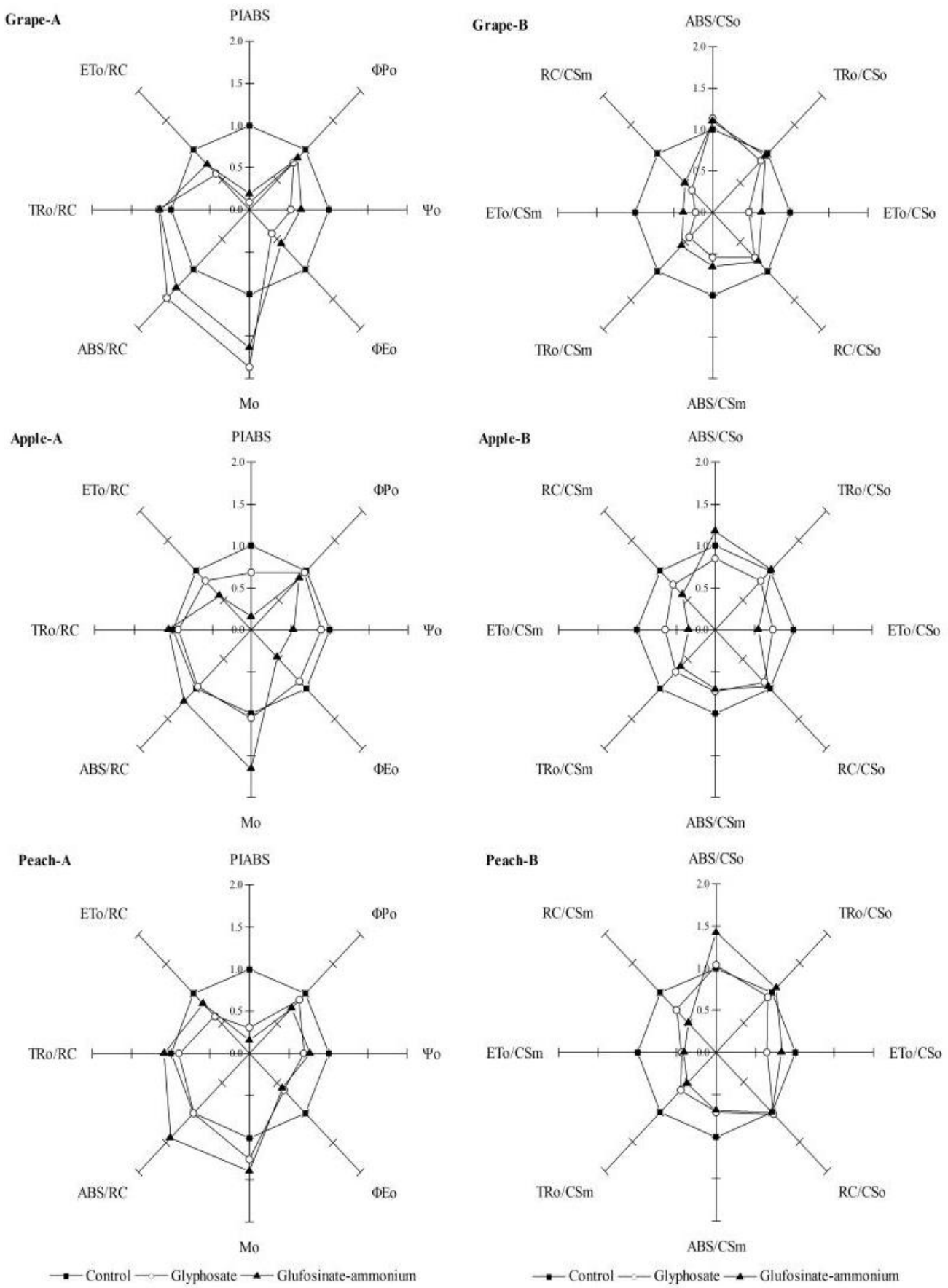

Fig. 5. Spider plots of the quantum efficiencies; flux ratios; ΦPo, ΦEo, and Ψ_O; the approximate initial slopes of the fluorescence transients (M_O values), the specific fluxes or specific activities (ABS/RC, TRo/RC, ETo/RC); the phenomenological fluxes or phenomenological activities (ABS/CS, TRo/CS, ETo/CS); the densities of reaction centers (RC/CS); and the performance indices (PI_{ABS} values) of leaves of various trees on the third day after herbicide applications.

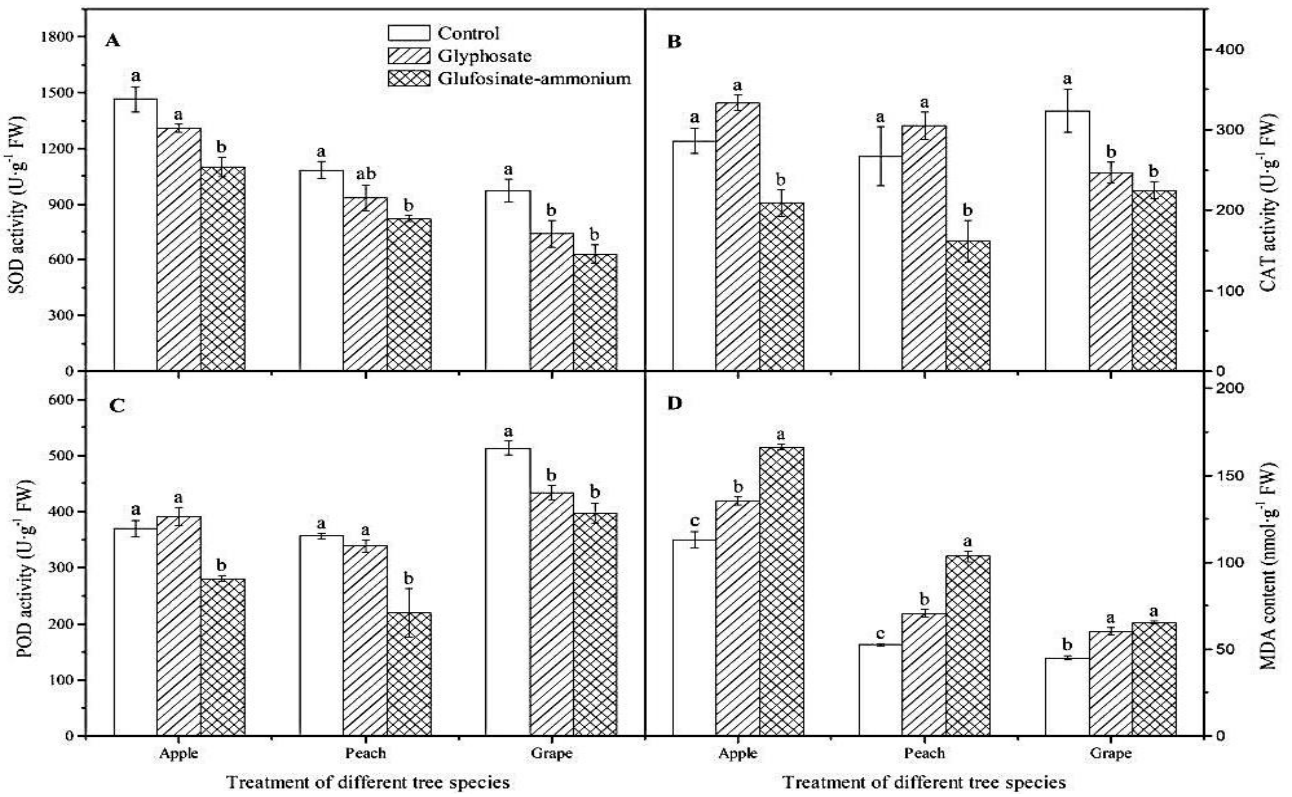

Fig. 6-1. The effects of glyphosate and GLA on superoxide dismutase (SOD; A), catalase (CAT; B), and peroxidase (POD; C) levels, and that of malondialdehyde (MDA; D) in leaves of various tree species on the third day. Different lower-case letters indicate significant differences between treatments ($p<0.05$). CAT activity (only) increased significantly (by 19.41% compared to the control) in apple leaves, whereas none of the SOD, POD, or CAT levels differed significantly from those of controls (Fig. 6-2A–C). However, the MDA levels of apple and peach leaves increased significantly (by 21.02% and 44.65%, respectively) (Fig. 6-2D). On the 19th day, the SOD, POD, and CAT activities decreased significantly (by 31.71%, 14.36%, and 22.47% in apple leaves and by 22.40%, 30.92%, and 25.60% in peach leaves) compared to controls (Fig. 6-2A–C) and the MDA levels were significantly higher than the control values (by 57.63% and 90.76%, respectively) (Fig. 6-2D).

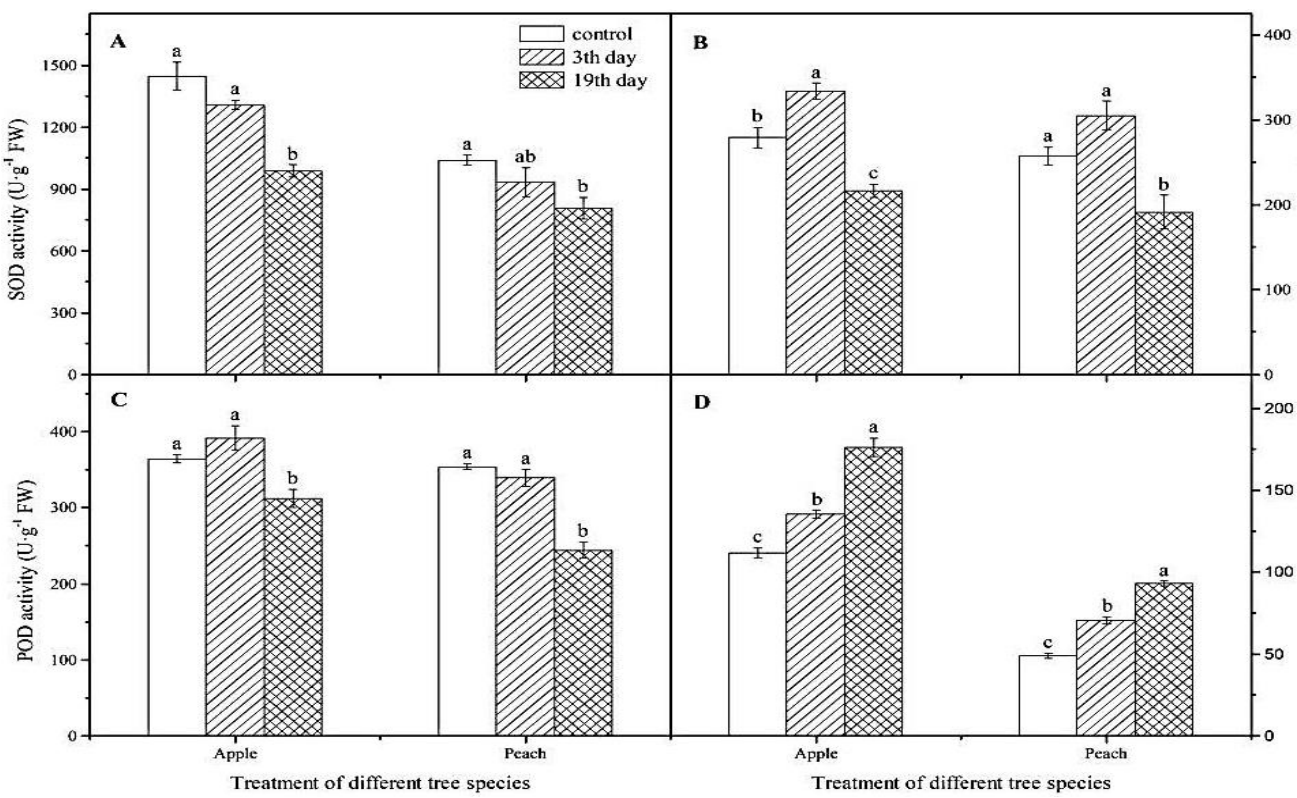

Fig. 6-2. Effects of glyphosate on superoxide dismutase (SOD; A), catalase (CAT; B), and peroxidase (POD; C) activities; and malondialdehyde (MDA; D) content in the leaves of different tree species on different days. Columns marked with different lower-case letters indicate a significant difference between treatments ($p<0.05$).

Conclusions

This study produced a number of interesting findings: (1) PSII activity fell after glyphosate and GLA application in three fruit trees, possibly associated with lower antioxidant enzymes activities and higher levels of MDA. (2) All species were less susceptible to glyphosate and more susceptible to GLA (the action modes of the two herbicides differ); apple was less susceptible than peach or grape. (3) No fruit tree recovered after glyphosate spraying, but apple and peach (not grape) recovered after GLA spraying. Orchardmen should thus reduce herbicide use or cover their fruit trees while spraying.

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