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.), ‘Chummi’ 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 PIABS 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 cells 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 (Gouglter & 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, non-selective, 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 (Fv/Fm) and photochemical quenching coefficient (Qe) (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 (Stajner 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,
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 (F0) and maximal fluorescence (Fm) were determined by turning on the measuring light (< 0.1 μmol·m−2·s−1) and the saturated pulse light (> 8,000 μmol·m−2·s−1), respectively (Maxwell & Johnson, 2000; Guo et al., 2005). Afterwards, the maximal fluorescence yield in the light-adapted state (Fm′) were determined by turning on the active light (538 μmol·m−2·s−1) and the saturated pulse light (> 8,000 μmol·m−2·s−1). When steady-state photosynthesis was attained, the fluorescence (Fv) was recorded, and then the minimal fluorescence yield of the light-adapted state (Fp′) was determined by turning on the far-red light (Van Kooten, 1990; Kanwal et al., 2011). The Fv/Fm, 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 μmol·m−2·s−1) (Tan et al., 2011). These transients were subjected to JIP testing using the following data: fluorescence intensity at 20 μs (F0); maximal fluorescence intensity (Fm); fluorescence intensity at 300 μs (the K-step) (F300μs); fluorescence intensity at 2 ms (the J-step) (Fj); and fluorescence intensity at 30 ms (the I-step) (Fj). 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·L⁻¹ potassium phosphate buffer (pH 7.8) containing 1 mmol·L⁻¹ EDTA and 1% (w/v) polyvinylpyrrolidone (PVP)]. Each homogenate was centrifuged at 15,000 r·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.

<table>
<thead>
<tr>
<th>Fluorescence parameter</th>
<th>Explanation</th>
<th>Formula</th>
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<tbody>
<tr>
<td>F₀</td>
<td>Steady-state fluorescence</td>
<td>F₀=Fₘ/ₐₔ</td>
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<tr>
<td>Fᵥ</td>
<td>Minimal fluorescence (dark)</td>
<td>Fᵥ=Fₘ/ₐₔ</td>
</tr>
<tr>
<td>Fᵢ</td>
<td>Fluorescence intensity at the I-step (30 ms)</td>
<td>Fᵢ=Fₘ/ₐₔ</td>
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<tr>
<td>Fₘ</td>
<td>Maximal fluorescence intensity</td>
<td>Fₘ=Fₘ/ₐₔ</td>
</tr>
<tr>
<td>Fᵢ</td>
<td>Relative variable fluorescence intensity</td>
<td>Fᵢ=Fₘ/ₐₔ</td>
</tr>
<tr>
<td>Vₒ</td>
<td>Relative variable fluorescence intensity at the J-step</td>
<td>Vₒ=Fₘ/ₐₔ</td>
</tr>
<tr>
<td>Mₒ</td>
<td>Approximate initial slope of the fluorescence transient</td>
<td>Mₒ=Fₘ/ₐₔ</td>
</tr>
<tr>
<td>Φₚₒ</td>
<td>Maximum quantum yield; primary photochemistry (at t=0)</td>
<td>Φₚₒ=Fₘ/ₐₔ</td>
</tr>
<tr>
<td>Ψₒ</td>
<td>Probability that a trapped excitation moves an electron into the electron transport chain beyond Qₒ (at t=0)</td>
<td>Ψₒ=Fₘ/ₐₔ</td>
</tr>
<tr>
<td>Φₑₒ</td>
<td>Quantum yield for electron transport (at t=0)</td>
<td>Φₑₒ=Fₘ/ₐₔ</td>
</tr>
<tr>
<td>Pₑₐₘ</td>
<td>Performance index on an absorption basis</td>
<td>Pₑₐₘ=(RC/ABS)·Φₑₒ/(1−Ψₒ)</td>
</tr>
<tr>
<td>Aₑₐₘ</td>
<td>Absorbed energy flux per reaction center (RC) (at t=0)</td>
<td>Aₑₐₘ=1/Fₑₒ</td>
</tr>
<tr>
<td>Tₑₐₘ</td>
<td>Trapped energy flux per RC (at t=0)</td>
<td>Tₑₐₘ=1/Fₑₒ</td>
</tr>
<tr>
<td>Eₑₐₘ</td>
<td>Electron transport flux per RC (at t=0)</td>
<td>Eₑₐₘ=1/Fₑₒ</td>
</tr>
<tr>
<td>Dₑₐₘ</td>
<td>Dissipated energy flux per RC (at t=0)</td>
<td>Dₑₐₘ=1/Fₑₒ</td>
</tr>
<tr>
<td>Aₑₐₘ</td>
<td>Absorbed energy flux per cross-section (CS)</td>
<td>Aₑₐₘ=1/Fₑₒ</td>
</tr>
<tr>
<td>Tₑₐₘ</td>
<td>Trapped energy flux per CS</td>
<td>Tₑₐₘ=1/Fₑₒ</td>
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<tr>
<td>Eₑₐₘ</td>
<td>Electron transport flux per CS</td>
<td>Eₑₐₘ=1/Fₑₒ</td>
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<td>Dₑₐₘ</td>
<td>Dissipated energy flux per CS</td>
<td>Dₑₐₘ=1/Fₑₒ</td>
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<tr>
<td>Rₑₐₘ</td>
<td>The proportion of active PSI reaction centers per excited CS</td>
<td>Rₑₐₘ=1/Fₑₒ</td>
</tr>
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Note: tFₘ=time to reach Fₘ (ms). When t>3, CSₒ becomes CSₘ, ABS/CSₘ=Fₘ
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 Fv/Fm 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 Fv/Fm 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 QP 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).
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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 F0 intensities to peak at P or Fm values when fronds were exposed to saturating actinic light. The F1 and F0 were sequentially present to between F0 and Fm. 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 PIABS 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 (MO 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, ΨO) 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 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).
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 $\Phi_{PSII}$ 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 μg·L$^{-1}$) observably decreased the $F_{v}/F_{m}$ ratio, the $\Phi_{PSII}$, 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 $\Phi_{PSII}$, 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; \( \Phi_\text{Po}, \Phi_\text{Eo}, \) and \( \Psi_\text{O}; \) the approximate initial slopes of the fluorescence transients (M\( \text{o} \) 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\text{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) PSIIL 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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References


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