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A comparison of the effects of acetochlor and fluoroglycofen on photosynthesis in grape leaves

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Abstract

Herbicides are widely applied to control weeds in vineyards. However, they may also impose abiotic stresses on non-target crops. Paraquat, acetochlor and fluoroglycofen are three types of herbicides that are sprayed onto the soil beneath grapevines after the fruit develops. The study aimed to characterize the effects of these herbicides in combination on the photosynthetic characteristics and nutrition storage of grapevine branches throughout the growing season in Qufu, Shandong, China. The results were compared to control vines treated with artificial weeding. The application of the three herbicides reduced the net photosynthesis rate (Pn) in a dose-dependent manner. At the highest concentration, the Pn was reduced by 34.6% 35 d after treatment. By 58 d, there was no significant difference between the lower concentration of herbicides and artificial weeding. Moreover, the combination of herbicides with the highest concentration of each reduced the free amino acid and soluble protein content by 10.4% and 15.1%, respectively, in the fourth node branches. The results of the pot experiment showed that acetochlor and fluoroglycofen both reduced Pn in dose-dependent manner. Moreover, both herbicides also enhanced the malonaldehyde content and accelerated the production rate of superoxide anions in grapevines. Taken together, these results suggested that acetochlor and fluoroglycofen produced different degrees of poisonous effects in grapevines. We suggest that orchard management strategies should reduce the number and dosage of herbicides, or cover crops instead.

Keywords: Herbicide; photosynthetic characteristics; grapevine.
Abbreviations: Gs, stomatal conductance; MDA, malondialdehyde; Pn, the net photosynthesis rate.

Introduction

Due to the continuous increase in agricultural production, herbicides are widely used to control weeds, and comprise over 65% of pesticides applied in the field (USDA, 1998). However, the massive introduction of these molecules into fields can generate long-term residues in food and the environment, which has led to increasing research attention into the adverse impact of pesticides in recent years. Aside from their impact on the environment and food quality, many herbicides adversely affect crop development, resulting in growth inhibition or delayed development, reductions in yields or germination percentages or tissue necrosis (Belgers et al., 2007; Bondada, 2011; Macedo et al., 2008; Robertson and Davis, 2011; Saladin et al., 2003a; Saladin et al., 2003b). Negative effects on grapevine growth and development have been shown after the application of classical herbicides including 2,4-D (Al-Khatib et al., 1993; Bondada, 2011), glyphosate (Ahsan et al., 2008), chlorsulfuron (Bhattia et al., 1998), diuron (Itoh and Manabe, 1997), trichloroacetate (Radetski et al., 2000), flazasulfuron (Magné et al., 2006) and flumioxazin (Bigot et al., 2007; Saladin et al., 2003a; Saladin et al., 2003b). Paraquat (1,1′-dimethyl-4,4′-bypiridilium) is one of the most widely used herbicides for the control of broad leaf weeds and grasses because of its excellent efficacy and low cost (Fuerst and Vaughn, 1990). Paraquat is absorbed very quickly by plant leaves, and it works as a competitive inhibitor of photosynthetic electron transport at the PS I level. Acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetamide] is a pre-emergence herbicide that is used to control broad leaf weeds and grasses; it belongs to the chloroacetanilide family of herbicides, and disrupts and inhibits photosynthetic electron transport (Huang and Xiong, 2009). Fluoroglycofen [O-[5-(2-chloro-α, α, α-trifluoro-of-toluene-oxy)-2-nitro-benzoyl]-hydroxy ethyl] is a post-emergence herbicide for the control of broad leaf weeds and grasses, which belongs to the diphenylethers family of herbicides. It acts by inhibiting protoporphyrinogenoxidase. In 2009, we created two vineyards that were exposed to paraquat, acetochlor or fluoroglycofen perennially. The grape leaves grew to be dark and round; however, the rate of photosynthesis decreased compared with the controls that were created with artificial weeding. Of particular note, plants exposed to higher levels of herbicides failed completely. In this study, we initially aimed to confirm the effects of the three herbicides on photosynthesis by grapevine leaves throughout the growing season, and then investigated and compared the adverse impacts of acetochlor and fluoroglycofen on grapevines in pot experiments.

Results

Effects of herbicide combination treatments on CO_2 assimilation and stomatal conductance of grape leaves in a vineyard

In the vineyard, we observed changes in the net photosynthetic rate (Pn) in the middle-node leaves throughout the growing season after the application of herbicides. As shown in Fig. 1a, the Pn in the middle-node leaves from vines treated with artificial weeding alone (C_1) initially increased by 17.9% after

23 d of herbicide treatment, and then decreased over time. Compared with C_1, the covered crops (C_2) initially had an improved Pn, which increased to its maximal level of 22.8% at 47 d after treatment. Thereafter, no significant effects on Pn were observed by 58 d after treatment. Lower concentrations of herbicides (T_1) caused a reduced Pn immediately after treatment, but this quickly returned to normal. With higher concentrations of herbicides (T_2), the Pn was reduced throughout the growing season to a maximum amount of 34.6% at 35 d after treatment. After treated for 58 d, there was no significant difference among the C_1, C_2 and T_1. However, combination herbicide treatment had few effects on stomatal conductance (Gs) (Fig. 1b).

Effects of combination herbicide treatments on the storage of nutrition by grape branches

The storage of nutrition in branches was measured after defoliation (Table 1). In a comparison with C_1 crops, covered crops (C_2) had significantly improved starch and soluble protein content by 22.7% and 33.6%, respectively; however, there was no significant effect observed regarding the content of soluble sugar and free amino acids. Starch and soluble protein content obviously increased after lower concentrations of paraquat, acetochlor and fluoroglycofen compared with C_1; however, compared with the C_2 plants, the soluble protein content decreased by 12.7%. Compared with the C_1 plants, higher levels of herbicide treatments reduced free amino acid and soluble protein content by 10.4% and 15.1%, respectively, and all storage nutrition parameters were obviously reduced compared with the C_2 plants.

Comparison of the effects of acetochlor and fluoroglycofen on CO_2 assimilation in grape leaves

In order to confirm that both acetochlor and fluoroglycofen produced poisonous effects on grapevines, potted experiments were conducted in the greenhouse. As shown in Fig. 2a, and 2b, the Pn in control leaves initially increased on day 6, and then decreased as time progressed; by day 30, it was 75.8% of its initial level at day 0. The application of acetochlor and fluoroglycofen reduced Pn in a dose-dependent manner, with the most obvious reductions found after treatment with the highest concentrations. By day 30, Pn was maximally decreased by 63.7% and 43.9%, respectively, compared with the baseline values; compared with the control, it decreased most by 39.5%, 19.7%, respectively. The Pn in the T_1 groups was only significantly lower than the controls at days 6 and 30; however, significant differences between the T_4 and control groups were only observed on day 12 post-treatment.

Comparison of the effects of acetochlor and fluoroglycofen on MDA content and superoxide production rate in grape leaves

As shown in Fig. 3a and 3b, the MDA content in control leaves on days 6 and 30 was significantly lower than the other three time points. Lower concentrations of acetochlor (T_1) first reduced the content of MDA compared with its initial level, while after that it returned to the baseline level. However, MDA content in the other two herbicide treatments (T_2 and T_3) first increased and then returned to the baseline level, albeit at a higher level than the controls; the highest increases were found on day 20 and day 12, at 52.3% and 99.3%, respectively. The effects of fluoroglycofen on MDA content were similar to those of acetochlor, with the exception that the highest increase in MDA content were found on day 30 and day 4, at 31.4% and 51.4% in T_5 and T_6 groups, respectively. The production rate of superoxide in the control leaves significantly increased by day 6, and then decreased to the level of the controls by day 12. After that, it increased as time progressed (Fig. 3c and 3d). Lower concentrations of acetochlor caused lower rates of superoxide production on day 6 and 30 compared to the control. Higher concentrations of acetochlor treatment caused a significant increase in the production of superoxide, with gains of 258.66% on day 12 post-treatment compared with the control; our results showed this rate reduced by day 20 and then increased again by day 30.

After fluoroglycofen treatment, superoxide production showed the same trend in variation as the control (Fig. 3d). Lower levels of fluoroglycofen reduced the superoxide production rate on days 6 and 12, and high levels of fluoroglycofen markedly increased the rate by day 6 post-treatment.

Discussion

In vineyards, various herbicides are widely applied to control weeds. Contact herbicides, such as paraquat, only result in the death of weeds if it comes into direct contact with its leaves, which rapidly absorb them. Acetochlor and fluoroglycofen can both be absorbed by both roots and leaves, upon which they enter the plant transport systems. In this current study, the application of a combination of paraquat, acetochlor and fluoroglycofen caused reduction of Pn in grapevines, especially at higher dosages. Our previous studies in two vineyards in Qufu, Shandong province, China, demonstrated a reduction in the grape yield compared with the artificial weeding controls of up to 13,500 kg ha^{-1}. The decrease of Pn was much higher in the earlier stages of treatment than in later stages, which could be attributed to herbicide degradation (Zhu et al., 2004; Wu et al., 2006), or the adaption of grapevines to them. Huang and Xiong (2009) suggested that cadmium (Cd), acetochlor (AC), bensulfuron-methyl (BSM) alone and their combination treatment led to a decrease in the soluble protein content in the shoots and roots of rice, but an increase in the level of free amino acids. Another protoporphyrinogen IX oxidase-flumioxazin herbicide induced an accumulation of soluble sugars in every grapevine organ (Saladin et al., 2003b). In this study, the application of higher level combined herbicides (paraquat, acetochlor and fluoroglycofen) reduced the content of soluble sugar, free amino acids and soluble protein of the fourth node branches. We hypothesized that this could have resulted from a lower Pn rate during the growing season. However, the individual effects of these herbicides on the soluble carbohydrate content of grapevines require further research. Our potted experiment results showed that acetochlor caused a greater reduction in the Pn than fluoroglycofen, which could be attributed to the different levels of oxidative damage caused by the two herbicides. Different classes of herbicides are either direct or indirect sources of oxidative damage in plants (Qian et al., 2008; Ramel et al., 2009). MDA is the final product of membrane lipid peroxidation, and its accumulation can be the result of ROS toxicity. Many studies have reported that numerous herbicides induced obvious accumulations of ROS and MDA (Alla et al., 2008; Hassan and Nemat Alla, 2005; Nemat Alla et al., 2008; Qian et al., 2008; Wu et al., 2010). Our results showed that both acetochlor and fluoroglycofen accelerated O_2^- production and resulted in the exacerbation of membrane lipid peroxidation, especially at higher herbicide concentrations, which might be related to lower antioxidant enzyme activities (Tan et al., 2012). The highest concentration of acetochlor caused the most significant increase in MDA content and O_2^- production rate at 12 d,

Table 1. Effects of herbicide treatments and artificial grasses on the sugar, starch, free amino acid and soluble protein contents of the fourth node branches of grapevines in a vineyard, Qufu City, China.

Treatments	Soluble sugar (mg g^{-1} FW)	Starch (%)	Free amino acid (NH_2-N μg g^{-1} FW)	Soluble protein (mg g^{-1} FW)
C_1	51.43±5.13 ab	5.10±0.52 b	6.45±0.42 a	20.85±0.65 c
C_2	53.67±0.92 a	6.26±0.49 a	7.01±0.23 a	27.85±1.09 a
T_1	51.02±0.56 ab	6.08±0.33 a	6.93±0.43 a	24.32±0.21 b
T_2	46.91±0.62 b	5.18±0.33 b	5.78±0.10 b	17.71±0.21 d

Values are the means ± SE of ten independent branches. Different small letters in the same row meant significant differences between treatments (P < 0.05).

compared to day 6 for the highest concentration of fluoroglycofen. All of the data suggest that the application of herbicides caused the accumulation of ROS and damaged the cell membranes. However, the response of the grapevines to acetochlor and fluoroglycofen differed, which may have results from the different durations of activity and transport routes of each herbicide. After pesticides enter the environment, their migration and transformation process is complex, and their negative effects on crops, including phytotoxicity, can be persistent. In 2009 and 2010, we observed three adjacent vineyards in Qufu, Shandong, China. One was treated with higher concentrations of paraquat, acetochlor and fluoroglycofen perennially, the combination of herbicides was applied four times in 2009, but in 2010, crops were covered. Even so, the Pn was lower than that of cover crops perennially, which might be attributed with the after effects of residual herbicides (Tan et al., 2011). In this current work, higher concentrations of acetochlor and fluoroglycofen inhibited grapevine growth in the second year, and the highest concentration of fluoroglycofen caused significant deformities of grapevine leaves. While many studies have suggested that covering crops improved the orchard ecological environment (Afonso et al., 2003; Monteiro and Lopes, 2007; Ramos et al., 2010; Sánchez et al., 2007) and fruit quality (Xi et al., 2011), this study showed that the leaf photosynthesis and storage nutrition of covered crop branches were enhanced compared to those treated with herbicides. We recommend that the application of herbicides should be limited or stopped, and crops should be covered instead.

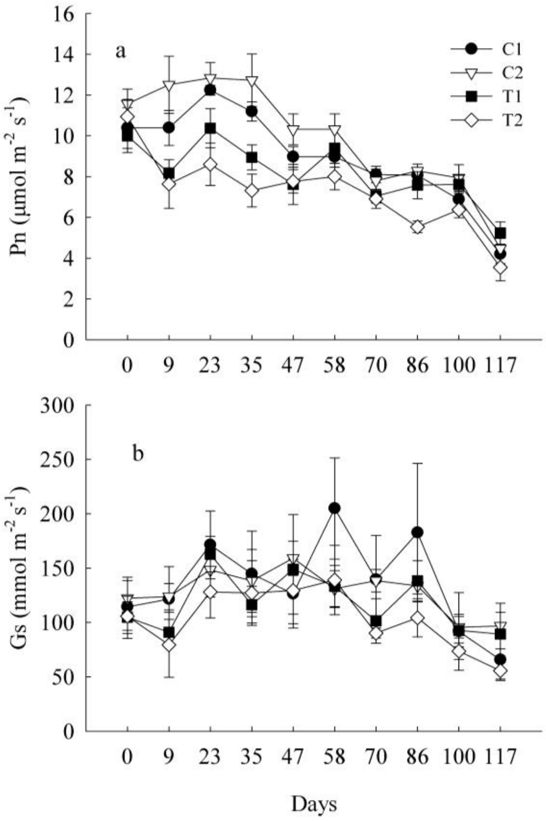

Fig 1. Effects of herbicide treatments on the net photosynthetic rate (Pn, a) and stomatal conductance (Gs, b) in middle-node grape leaves. The values are shown as the means ± SE of less than ten independent grapevines.

Materials and methods

Plants, growth conditions and herbicide treatments

Experiment 1 was conducted in a vineyard planted with 10-year-old Kyoho plants (*V. vinifera* × *V. labrusca*) in Qufu city, Shandong province, China, in 2010. The rows and vines measured 1.6 x 0.8 m, respectively, and the rows were oriented in a north-south direction. Vines were trained to two trunks and a bilateral cordon. Over the past 10 years, the management measures have been consistent. Four treatments were established with two replicates; each replicate (plot) had three rows with 40 vines per row. The treatments were as follows: (1) C_1, artificial weeding between rows; (2) C_2, crop were covered between the rows with white clover (*Trifolium repens* L.); (3) T_1, herbicides were applied on the 20th June and the 26th July, using a combination of 1,500 ml ha^{-1} acetochlor (99.9%), 150 ml ha^{-1} fluoroglycofen (10%) and 1,500 ml ha^{-1} paraquat (10%); (4) T_2, herbicides were applied twice (on June 20th and July 26th) using a combination of 2,250 ml ha^{-1} acetochlor (99.9%), 225 ml ha^{-1} fluoroglycofen (10%) and 2,250 ml ha^{-1} paraquat (10%). After the grapevines were treated for 0, 9, 23, 47, 70, 86, 100 and 117 days (all were fine weather days), less than five grapevines in the two replicates were selected to test gas exchange in the middle-node (8/9) leaves. After defoliation, the storage nutrition contents in the fourth nodes branches were also measured. Experiment 2 was conducted at the greenhouse in Shandong Agriculture University, China, in 2010. One-year old grapevines (*V. vinifera* × *V. labrusca* cv. Kyoho) were grown in 25.0-cm-diameter plastic pots containing garden earth, sand, and matrix soil (2:1:1) at approximately 25-28°C in the greenhouse with PPFD of 400 μmol m^{-2} s^{-1}, a relative humidity of 75%-80%, and a photoperiod of 14/10 h light/dark. When the shoots had ten leaves, acetochlor or fluoroglycofen was sprayed a single time 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); and (6) T_6, 375 g ai ha^{-1} fluoroglycofen (0.00230 g ai per pot). Simultaneously, the soil of the control plants was sprayed with water. The experiment was performed twice with three plants in each treatment group. After the grapevines had been exposed to herbicide for 0, 6, 12, 20 and 30 days, physiological indices

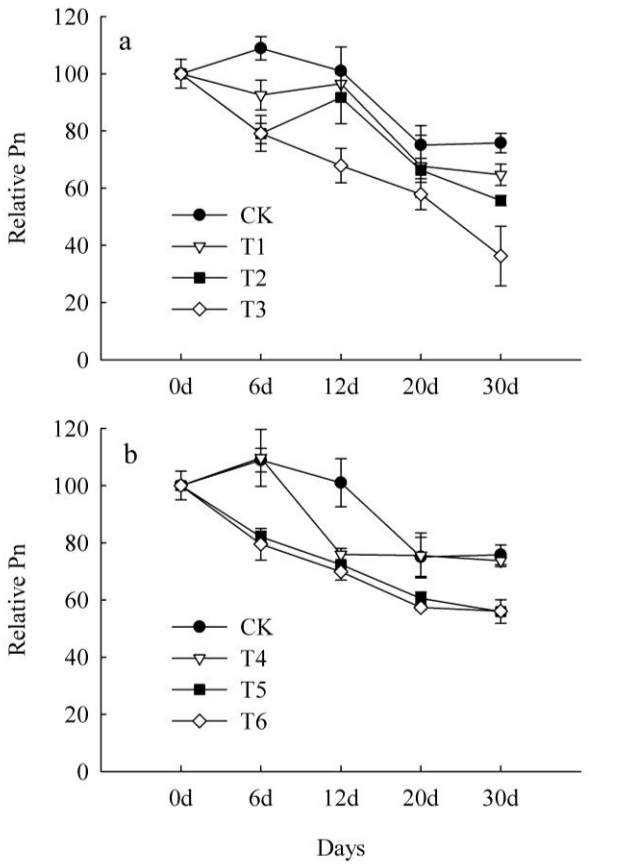

Fig 2. Effects of different concentrations of acetochlor (a) and fluoroglycofen (b) treatments on relative net photosynthetic rate (Pn) in middle-node leaves in annual grape seedlings. The level of control was set as 100 at 0 d. The values are shown as the means ± SE of six independent grapevines.

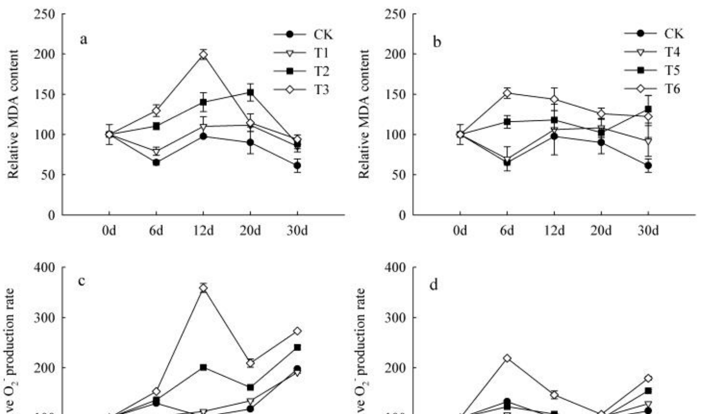

Fig 3. Effects of different concentrations of acetochlor (a, c) and fluoroglycofen (b, d) treatments on the relative MDA content (a, b) and relative $O_2^{\cdot -}$ production rate (c, d) in middle-node leaves in the annual grape. The level of control was set as 100 at 0 d. The values are shown as the means ± SE of six independent grapevines.

were analyzed in the middle-node (8/9) leaves in grape seedlings. The baseline control level was set as 100 at 0 d, and all other results were compared to this level.

Gas exchange measurements

Measurements of net photosynthetic gas exchange were made on the middle nodes leaves of grape seedlings using an open system (Ciras-2, PP Systems, Hitchin, UK).

Assays of storage nutrition in branches

Total free amino acid contents in shoots were estimated by the ninhydrin method, using leucine as standard by the method according to (Xiong et al., 2006). Total free amino acids were expressed as µg free amino-N g^{-1} FW. The protein content of the tissue extracts was determined according to the method of (Bradford, 1976), using bovine albumin as the standard. Fresh branches were used to measure starch and soluble sugar content. 0.5 g branches were boiled in glass tubes containing 20 ml distilled water. After filtering, the supernatants were volumed to 50 ml, and used for total soluble sugar determination whereas the pellets were kept for starch analysis. Total soluble sugars: a 0.2 ml of the supernatant was mixed with 1 ml of anthrone–sulfuric reagent (0.1% anthrone and 0.1% thiourea in 12.5 N sulfuric acid) and incubated for 10 min at 100 ^oC. After cooling, the absorbance was read at 625 nm (Yemm and Willis, 1954) and results were expressed in mg of glucose equivalents per g of fresh weight. Starch：The pellet collected following the extraction of total soluble sugars was resuspended in 25 ml of distilled water, containing 2 ml 9.2 mol L^{-1} perchloric acid solution . Starch was dissolved for 30 min at 100 ^oC. After centrifugation for 10 min at 2500 g, 0.5 ml of the supernatant was mixed with 1 ml of anthrone–sulfuric reagent (0.1% anthrone and 0.1% thiourea in 12.5 N sulfuric acid) and incubated for 10 min at 100 ^oC. After cooling, the absorbance was read at 630 nm.

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 (Heath and Packer, 1968). The production rate of O_2^- was determined according to (Elstner and Heupel, 1976) by monitoring the nitrite formation from hydroxylamine in the presence of O_2^-.

Statistical analysis

Each reported data are the mean ± standard error (SE) of all 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 probability level of 0.05.

Conclusion

We conclude from the present findings that the reduction in nutrition storage in branches could be attributed to a decrease in photosynthesis in the growing season after the application of paraquat, acetochlor and fluoroglycofen to vineyards. Furthermore, high concentrations of acetochlor and fluoroglycofen reduced the rate of photosynthesis to different extents, which could be related to different degrees of oxidative damage caused by the two herbicides.

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