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Photosynthesis Limitations of Grapevine after Treatment with the Fungicide Fludioxonil

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The aim of this work was to determine the major limitations to photosynthesis induced by the fungicide fludioxonil (fdx) on nontarget grapevines using cutting as a model. The fdx treatments (1.2, 6, and 30 mM) induced a net photosynthetic rate (P_n) decrease without changes in stomatal conductance, suggesting a nonstomatal limitation. Fdx effects on P_n were related neither to photosynthetic capacity alteration in leaves nor to loss in PSII activity. The mechanism underlying photosynthesis reduction differed according to the concentration. Fdx at 6 mM led to an increase of light requirement for photosynthesis while 30 mM fdx induced an increase in the respiration rate in the light. P_n decrease after 1.2 mM fdx could rather be related to wetness caused by the spraying than to fungicide toxicity. P_n recovered 10 days after treatment, meaning that fdx had little deleterious effect on plant physiology or that grapevine has a great capacity to overcome this temporary stress.

KEYWORDS: Chlorophyll fluorescence; fludioxonil; fungicide; gas exchanges; grapevine; phytotoxicity

INTRODUCTION

In vineyards, productivity requires several pesticide treatments because of the susceptibility of grapevine to a range of diseases. Especially, fungal pathogens are a major problem in the cultivation of grapevine (*Vitis vinifera* L.) around the world. Thus, fungicides represent 80% of all pesticides used in vineyards (*1*). Among them, botryticides are used to control *Botrytis cinerea*, the causal agent of gray mold disease, which causes worldwide yield loss (*2*). Chemical control currently remains the main way to fight this phytopathogenic fungus. Three preventive applications are usually recommended: at the end of flowering (BBCH 69), at bunch closure (BBCH 77), and at the beginning of berry ripening (BBCH 81). Fludioxonil (fdx) [4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1*H*-pyrrole-3-carbonitrile], a phenylpyrrole compound, is commonly used as a botryticide all over the world (*3*). Fdx inhibits spore germination, germ-tube elongation, and the mycelium growth of *B. cinerea* (*4*). It increases the glycerol content in the fungus, leading to a perturbation of the osmoregulation potential (*5*).

Considerable use of pesticides in vineyards generates long-term residues in food and the environment. In addition, some pesticides may also have consequences on crop physiology, such as growth reduction, perturbation of reproductive organ development, alteration of nitrogen, and/or carbon metabolism (*6*). This former physiological trait is fundamental for crop culture and is reflected by both photosynthetic rate and mobilization of carbohydrate reserves. Indeed, as plants rely on their ability to assimilate carbon through photosynthesis for their growth and overall vigor, photosynthesis disruption may decrease yield and vigor. Several works on photosynthesis fluctuations after fungicide application on various crops report modifications of both photosynthetic activity and chlorophyll fluorescence (*7–10*). Photosynthesis alteration was revealed by reduction in net photosynthesis accompanied by changes in stomatal conductance and intercellular CO_2 concentration (*8–10*). Modifications of dark respiration were also noticed after fungicide treatment (*9*). Considering fluorescence, the relative quantum yield of PSII (Φ_{PSII}) and the maximal quantum efficiency of PSII (F_v/F_m) were reduced by some fungicides and were attributed to decrease in photochememical quenching (q_P) (*7, 10*).

Fdx impacts on photosynthesis have already been shown to modify CO_2 fixation and photosynthetic pigment concentration after application on grapevine leaves (*11*). The decrease in CO_2 fixation after fdx application may be attributed to stomatal closure, disruption in capacity of rubisco carboxylation and/or RuBP regeneration, and loss in photosystem II (PSII) activity. The aim of this study was therefore to determine changes in photosynthetic performance and to localize primary sites of damage following fdx application. Different aspects of leaf photosynthesis were thus determined on fruiting cuttings of *V. vinifera* L. (cv. Pinot noir) after fdx treatment at the end of flowering (BBCH 69). At this time, mature leaves are the main sources of carbohydrates for developing leaves, roots, flowers, and berries (*12*). Any perturbation during flower development may thus lead to a decrease in fertilization and yield (*13*). Analyses of gas exchanges with photosynthesis versus inter-

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cellular CO_2 concentration and light response curves allowed evaluation of relative limitations imposed by stomata, carboxylation efficiency, and capacity of RuBP regeneration on leaf photosynthesis (*14–18*). Light respiration has also been investigated to study its putative implication in photosynthesis decrease. Chlorophyll florescence parameters were also analyzed because they are reliable indicators of photosynthetic apparatus state (*19–22*). Finally, Hill reaction activity, which reflects PSII integrity, as well as the efficiency of electron transport, has been evaluated.

MATERIALS AND METHODS

Plant Material. Fruiting cuttings of *V. vinifera* L. (cv. Pinot noir) were used for this experiment. Fruiting cuttings were obtained from canes of grapevine according to the protocol improved by Lebon et al. (*23*). Cuttings were planted in 300 mL pots containing a perlite/sand mixture (1:2, v/v) and transferred to a growth chamber under a temperature of 25 °C (day/night), at a relative humidity of 60% (day/night) and a 16 h photoperiod (400 μmol photons m^{-2} s^{-1}). Plants were irrigated daily with a Coïc and Lesaint medium (*24*). The fdx solution was sprayed after 6 weeks of growth, when cuttings had four leaves and an inflorescence at the end of flowering (BBCH 69). Fdx ($C_{12}H_6F_2N_2O_2$) was obtained from the commercial fungicide Géoxe. The molecular mass of fdx is 248.2 and its water solubility is 1.8 mg L^{-1}. Treatment was performed once with different solutions of fdx in water, 1.2, 6, or 30 mM, corresponding to 0.2, 1, and 5 times the concentration recommended by the manufacturer, respectively. Controls were carried out using untreated plants or plants sprayed with water.

Leaf Gas Exchanges. The net photosynthetic rate (P_n), the stomatal conductance (g_S), and the intercellular CO_2 concentration (C_i) were measured with an open gas exchange system (LI-6400, Li-Cor, Lincoln, NE) using equations developed by Von Caemmerer and Farquhar (*25*). The infrared gas analysis system was equipped with a clamp-on leaf cuvette that exposed 6 cm^2 of leaf area. Air temperature and humidity were maintained at 25 °C and 30%, respectively. Photosynthetically active radiation provided by a red−blue light emitting diode (Li-6400-02, Li-Cor) was fixed at 1500 μmol m^{-2} s^{-1}. Carbon dioxide concentration (C_a) was maintained at a constant level of 400 μmol L^{-1} using a CO_2 injector with a high-pressure liquid CO_2 cartridge source (LI-6400-01, Li-Cor). The same leaves were used during all the kinetic analysis corresponding to a few hours before fungicide application and then 1, 2, 4, 7, 10, and 14 days after treatment. Gas exchange measurements were performed on eight leaves of different plants and three times per leaf. The second leaf from the base of each plant was chosen for measurements.

Photosynthesis response curves to varying C_i (P_n/C_i) were determined at a saturating photosynthetic photon flux density (PPFD) by step changes of 12 new C_a from 0 to 2000 μmol L^{-1}. Gas exchange measurements were determined at each step after P_n stabilization. Measurements and interpretation of the P_n/C_i response have been described previously by Long and Bernacchi (*26*). The in vivo maximum rate of rubisco carboxylation ($V_{c,max}$) was estimated as the slope of the linear portion of CO_2 response curve from the asymptote of the fitted response function (*27*). C_i was regarded as the CO_2 compensation point (C_i*) when P_n was zero, and P_n was estimated as the mitochondrial respiration in the light (R_l) when C_i was zero.

The responses of P_n to step changes in PPFD was measured at a constant C_a of 360 μmol L^{-1}. Twelve PPFDs from 0 to 2000 μmol photons m^{-2} s^{-1} were set. The apparent quantum yield of CO_2 fixation (ΦCO_2) was estimated as the slope of the linear portion of the PPFD response curve. ΦCO_2 is the efficiency of light use in photosynthesis, i.e., the number of moles of CO_2 fixed per mole quantum absorbed by a leaf. The ratio $\Phi_{PSII}/\Phi CO_2$ was also calculated; it represents an estimate of the relationship between the rate of electron transport and carbon fixation. Moreover, the light compensation point (C_i*) and dark respiration (R_d) were calculated from the response curves according to Long and Hällgren (*28*).

Leaf intercellular CO_2 concentration saturated assimilation rate ($Pmax_{(Ci)}$) and PPFD-saturated CO_2 assimilation rate ($Pmax_{(PPFD)}$) were estimated from the irradiance response and CO_2 response curves, respectively. Curves were obtained from three plants per treatment. P_n/C_i and photosynthetic light response curves were done 7 days after treatment.

Chlorophyll *a* Fluorescence. Chlorophyll *a* fluorescence was quantified on attached leaves with a chlorophyll fluorescence imaging system (IMAGING-PAM, Walz, Effeltrich, Germany). The measuring system applied an array of blue-light-emitting diodes (peak wavelength, 470 nm) for saturating light pulses. The frequency of the pulses was adjusted to 10 Hz. Measurements were carried out at a maximal distance between the camera and the leaf, corresponding to a 25 × 34 mm area. The image captured by the charge coupled device (CCD) camera was composed of 640 × 480 pixels. During the whole experiment, measurements were systematically performed on the adaxial side on the central parts of leaves. Leaves were dark adapted for at least 30 min to determine the (F_0) (minimal) level of fluorescence and the maximal fluorescence (F_m) after a saturating flash (1 s, 13 000 μmol m^{-2} s^{-1}). During actinic illumination, chlorophyll fluorescence measurements were taken continuously (F_t). After stabilization, gas exchanges were determined followed by a saturating flash of 2 s duration to measure the maximal fluorescence of a light adapted leaf (F_m'). Removal of the actinic light and the presence of a short period of far-red light allowed measurement of the zero level of fluorescence (F_0'). From these measurements, several fluorescence parameters were calculated according to Schreiber et al. (*29*) and Genty et al. (*22*): $\Phi_{PSII} = F_m' - F_t/F_m'$ and $F_v/F_m = (F_m/F_0)/F_m$. Φ_{PSII} represents the number of electrons transported by a PSII reaction center per mole of quanta absorbed by PSII and F_v/F_m is the ratio of variable to maximal fluorescence. In addition, both photochemical (q_P) and nonphotochemical quenching (q_{NP}) were calculated according to Van Kooten and Snel (*30*): $q_P = (F_m' - F_t)/(F_m' - F_0')$ and $q_{NP} = (F_m - F_m')/F_m'$. q_P reflects the number of open reaction centers. It is an indicator of the capacity of photochemical processes. q_{NP} is linearly related to heat dissipation and is the most common form of protection against excess photons (*19, 20*). On each image, the values of the selected fluorescence parameters were averaged. Images of Φ_{PSII} were displayed with the help of a false color code ranging from 0.000 (black) to 1.000 (pink). Chlorophyll *a* fluorescence measurements were performed on the same leaves and according to the same kinetic as the gas exchanges.

Chloroplasts Isolation and Hill Reaction Measurements. Seven days after treatment, one leaf per plant was collected. The protocol of chloroplasts isolation was modified according to Hernández-Gil and Schaedle (*31*). One gram of leaves was quickly homogenized in a cooled mortar with Fontainebleau sand in 10 mL of extraction medium consisting of 0.35 M sorbitol, 50 mM tricine, pH 7.6, 2 mM EDTA, and 1.5% polyethylene glycol (PEG) 4000 (w/v). The chloroplast homogenate was filtered through fine nylon net. The filtrate was centrifuged for 60 s at 2500*g*. The supernatant fluid was discarded and the pellet containing whole chloroplasts was resuspended in a small volume of homogenizing buffer. At this point, the chloroplast suspension was stored in an ice-cooled glass beaker in the dark. Total chlorophyll (chl) concentration was determined in 80% acetone as described by Lichtenthaler et al. (*32*). The reaction mixture containing chloroplasts equivalent to 20 μg of chl mL^{-1} was used to determine Hill reaction activity.

The rate of the Hill reaction was determined in chloroplast by following the rate of 2,6-dichlorophenol-indophenol (2,6-DCPIP) photoreduction using spectrophotometer at 600 nm. As the rate of oxygen release parallels the rate of DCPIP reduction, results were expressed in mmol O_2 mg of chl^{-1} h^{-1}. Four plants for each treatment were used in this experiment.

Statistical Analysis. To determine whether values of untreated plants were significantly different from treated plants, analysis of variance (ANOVA) followed by a Student's *t* test was used. Differences at $P < 0.05$ were considered significant.

RESULTS

Gas Exchanges. The first day after treatment, significant P_n decrease was observed using water and 1.2 mM fdx treated plants (**Figure 1a**). P_n was inhibited by about 30% after both

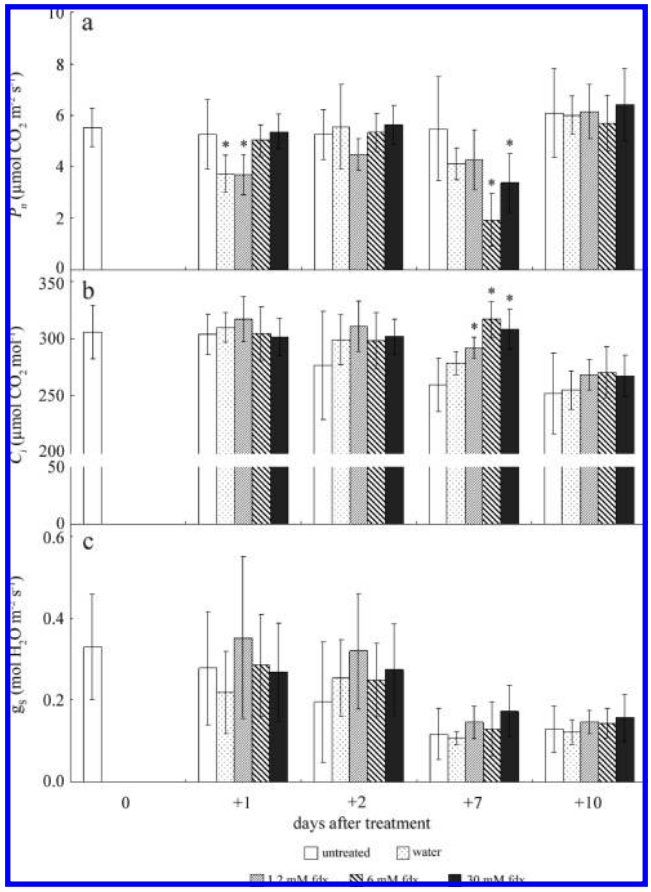

Figure 1. Changes in (**a**) net photosynthesis (P_n), (**b**) intercellular CO_2 concentration (C_i), and (**c**) stomatal conductance (g_S) in untreated, water-treated, and fdx-treated leaves of grapevine. Data are means ± standards errors ($n = 24$). Significant differences at $P < 0.05$ between leaves of untreated and treated plants are marked by an asterisk.

treatments, while it was not significantly different with 6 and 30 mM fdx application. However, g_S and C_i remained stable (**Figure 1b,c**). After 2 and 4 days of treatment (data not shown), the rate of P_n was not significantly different between controls and treated plants. Nevertheless, 6 and 30 mM fdx treated plants had significantly lower P_n than those measured in untreated plants 7 days after treatment. The reduction represented 38 ± 21% of the control values in 30 mM fdx treated plants. P_n decrease was higher with 6 mM fdx since it represented 65 ± 19%. In both cases, P_n reduction was accompanied by increased C_i without modification of g_S. The rise of C_i represented approximately 20% after both treatments. Even if P_n was not modified in 1.2 mM fdx treated plants, C_i increased by 13 ± 4%. Ten days after treatment, gas exchanges recovered in all treated plants and remained stable 14 days after treatment (data not shown).

P_n/C_i and light response curves were used in order to clarify the mechanisms involved in photosynthesis limitations following treatments. This study was performed 7 days after treatment because treated plants presented dramatic photosynthesis disruptions at this time. P_n/C_i curves showed that neither $Pmax_{(Ci)}$ nor $V_{c,max}$ were affected by water or fdx treatments (**Table 1**). Nevertheless, C_i* and R_l strongly increased after water and 30 mM fdx treatments. In detail, C_i* and R_l were enhanced after water treatment by 2.6- and 2.3-fold, respectively. Changes were significantly higher with 30 mM fdx: this treatment generated a 3.3- and 3.5-fold increases of C_i* and R_l, respectively.

In complement, analysis of light response curves was performed. The ΦCO_2 value significantly decreased after all fdx treatments (**Table 2**). ΦCO_2 decline represented approximately 30−45% according to the treatment. In 1.2 mM fdx treated plants, R_d decreased by half. Γ* was affected by 20% in 6 mM fdx treated plants, but no significant differences were registered between untreated and the other treated plants. Considering $Pmax_{(PPFD)}$, no significant changes were detected whatsoever after treatment. The $\Phi_{PSII}/\Phi CO_2$ ratio was inversely correlated to the efficiency of light involvement for carbon fixation. It was significantly higher in all treated plants, indicating that light was less efficient after treatment using either water or fdx. $\Phi_{PSII}/\Phi CO_2$ increase represented from 50 to 90% according to the treatment.

Chlorophyll *a* Fluorescence. All treated plants had lower relative quantum yield of PSII (Φ_{PSII}) values than those measured in untreated plants, only 2 days after treatment (**Figure 2**a). The reduction reached approximately 10% in water, 1.2 mM and 30 mM fdx-treated plants and 7% in 6 mM fdx-treated plants.

Analysis of fluorescence showed a decrease in fluorescence emission, when cuttings were exposed to both water and fdx treatments (**Figure 3**). Modifications appeared throughout the mesophyll for all treatments. The maximum efficiency of PSII photochemistry after dark-adaptation (F_v/F_m), photochemical quenching (q_P), and total nonphotochemical quenching (q_{NP}) were not affected by various treatments during the whole experiment (**Figure 2b−d**).

Hill Reaction Activity. Compared to untreated plants, Hill reaction activity significantly decreased by 36 ± 13% 7 days after 6 mM fdx treatment (**Figure 4**). Using the other concentrations, no significant changes were noticed.

DISCUSSION

The results of the present study showed that photosynthesis was temporarily inhibited after the three fdx concentrations used and also after water application. The impact of fdx on grapevine photosynthesis was different according to the applied concentration. Both water and the lower fdx concentration (1.2 mM fdx) decreased photosynthesis from the first day following treatment. On the contrary, the higher concentrations, 6 and 30 mM, induced P_n decrease after 7 days. Light compensation point and respiration in the light increased 7 days after 6 and 30 mM fdx treatments, respectively. At 10 days after fdx application, recovery of P_n was observed in all treated plants.

As shown by P_n declines, fdx disrupted photosynthesis when applied at the highest concentrations (6 and 30 mM). This information is consistent with previous results obtained in grapevine (*11*) and in other plants with other fungicides (*8, 10*). In addition, fdx phytotoxicity varies according to its applied concentration on grapevine leaves, as observed with other fungicides. For example, the effect of captan on pepper and carbendazim on tobacco revealed that pigment reduction was more pronounced at higher concentrations (*33, 34*). In our experiment, the mechanism underlying photosynthesis inhibition also varies according to fdx concentration. Photosynthesis disruption after both water and 1.2 mM fdx sprayings was similar. P_n was impaired very quickly. Indeed, P_n disruption was detected as early as 1 day after treatment, then P_n recovered from the second day. P_n decrease was not related to changes in both g_S and C_i for both treatments, suggesting a nonstomatal limitation. It seems that loss in PSII activity is not involved in the P_n decrease after water and 1.2 mM fdx. Indeed, Φ_{PSII} reduction was only observed 2

Table 1. Determination of P_n/C_i Response Curves Parameters—$Pmax_{(Ci)}$, $V_{c,max}$, C_i^*, and R_l—in Grapevine Leaves of Untreated, Water-Treated, and Fdx-Treated Plants 7 days after Fdx Application[a]

			fdx-treated		
	untreated	water-treated	1.2 mM	6 mM	30 mM
$Pmax_{(Ci)}$ (μmol CO_2 m^{-2} s^{-1})	11.3 ± 0.8a	10.9 ± 1.3a	11.3 ± 0.1a	11.5 ± 0.9a	12.2 ± 1.1a
$V_{c,max}$ (μmol CO_2 m^{-2} s^{-1})	42.8 ± 3.2a	40.1 ± 10.1a	38.0 ± 3.2a	44.1 ± 2.8a	46.7 ± 5.4a
C_i^* (μmol CO_2 mol^{-1})	74.8 ± 12.7c	190.8 ± 37.7b	84.9 ± 10.3c	82.2 ± 13.9c	267.0 ± 8.5a
R_l (μmol CO_2 m^{-2} s^{-1})	3.2 ± 0.5c	7.5 ± 0.4b	3.2 ± 0.1c	3.6 ± 0.9c	12.6 ± 1.1a

[a] $Pmax_{(Ci)}$, intercellular CO_2 concentration-saturated net CO_2 assimilation rate; $V_{c,max}$, in vivo maximum rate of rubisco carboxylation; C_i^*, CO_2 compensation point; and R_l, estimation of mitochondrial respiration in the light. Data are means ± standards errors ($n = 3$). Means for a considered parameter were not significantly different, when followed by the same letter ($P < 0.05$).

Table 2. Analyses of Photosynthetic Light Response Curve—ΦCO_2, R_d, $Pmax_{(PPFD)}$, and $\Phi_{PSII}/\Phi CO_2$ Ratio—of Grapevine Leaves of Untreated, Water-Treated and Fdx-Treated Plants 7 Days After Fdx Application

			fdx-treated		
	untreated	water-treated	1.2 mM	6 mM	30 mM
ΦCO_2	0.07 ± 0.01a	0.05 ± 0.01a	0.04 ± 0.00b	0.04 ± 0.01b	0.04 ± 0.01b
R_d (μmol CO_2 m^{-2} s^{-1})	1.6 ± 0.0a	1.1 ± 0.1b	0.8 ± 0.0c	1.2 ± 0.1b	1.4 ± 0.2ab
Γ^* (μmol CO_2 mol^{-1})	22.6 ± 3.1cb	26.1 ± 4.1ac	19.8 ± 1.1a	27.7 ± 1.4a	37.0 ± 15.8ac
$Pmax_{(PPFD)}$ (μmol CO_2 m^{-2} s^{-1})	8.9 ± 1.5a	7.7 ± 0.2a	7.7 ± 1.5a	7.6 ± 1.8a	9.4 ± 0.2a
$\Phi_{PSII}/\Phi CO_2$	10.0 ± 1.7b	15.0 ± 2.3a	17.4 ± 0.1a	16.3 ± 2.8a	19.2 ± 5.6a

ΦCO_2, apparent quantum yield of CO_2 fixation; R_d, dark respiration; Γ^*, light compensation point; and $Pmax_{(PPFD)}$, PPFD-saturated net CO_2 assimilation rate. Data are means ± standards errors (n=3). Means for a considered parameter were not significantly different, when followed by the same letter ($P < 0.05$).

days after treatment, whereas P_n was not lower in water and 1.2 mM fdx treated plants compared to untreated plants. Therefore, water and 1.2 mM fdx decreased P_n but did not significantly affect Φ_{PSII} the first day following application, suggesting that the rate of noncyclic electron transport is higher than that required to maintain P_n. An alternative sink to P_n for electrons may be oxygen reduction by photorespiration, Mehler ascorbate peroxidase reaction, mitochondrial respiration in the light, and/or dark respiration (*35*). Nevertheless, R_l was not increased after 1.2 mM fdx treatment, excluding the hypothesis that light respiration increase may contribute to P_n decrease. In addition, analysis of chlorophyll fluorescence was also performed, since it is frequently used to monitor responses of photosynthetic apparatus to environmental stress (*19, 20*). Water and 1.2 mM fdx did not affect q_P and q_{NP}, indicating that energy dissipation was not modified by these treatments. This suggests that the nonstomatal limitation of P_n after water and 1.2 mM fdx may originate from a decrease in CO_2 fixation mediated by rubisco. Indeed, the extent of P_n decrease after both treatments is similar, suggesting that P_n alteration induced by 1.2 mM fdx might be mostly attributed to wetness on leaf surface caused by spraying and not by toxicity of the active ingredient. This hypothesis is supported by previous works. An inhibition of P_n was actually observed in bean leaves in response to wetness (*36*) and was accompanied by a strong degradation of rubisco. Decrease in rubisco amount could thus explain the P_n decrease after 1.2 mM fdx. Our results also indicate that water spraying often used as control in studies of pesticide toxicity (*10, 34, 37*) is not well adapted because of photosynthesis modification caused by water.

The P_n disturbance induced by higher fdx concentrations, 6 and 30 mM fdx, is very different than the one caused by both water and 1.2 mM fdx. At 6 and 30 mM fdx, P_n inhibition at 7 days is associated with increased C_i, indicating a nonstomatal limitation. The strongest effect was registered after a 6 mM fdx treatment. It is likely that PSII is not involved in P_n reduction after both treatments because Φ_{PSII} reduction was only observed 2 days after treatment and recovered rapidly the day after. This may further reflect the ability of treated leaves to maintain high electron transport rates. Many abiotic stresses such as drought, salinity stress, and herbicide application are known to induce decreases in $V_{c,max}$ and $Pmax_{(Ci)}$(*14, 17, 38, 39, 40*). Nevertheless, measurements of P_n versus C_i revealed that these gas exchange parameters were not reduced after fdx treatments. This indicates neither loss nor inactivation of both rubisco and other key Calvin cycle enzymes, which may result in a reduction of carboxylation efficiency and RuBP regeneration rate (*41, 42*). In agreement with these observations, $Pmax_{(PPFD)}$ was also not altered after treatments, suggesting that neither maximal rubisco activity nor RuBP regeneration were modified (*27*). Hence, the photosynthetic capacity of leaves does not seem be affected by fdx application. Reduction in activity of other enzymes, such as carbonic anhydrase, which catalyzes the conversion of CO_2 to HCO_3, or enzymes involved in the utilization of the photoassimilates, may thus cause a decrease in CO_2 fixation. Indeed, carbonic anhydrase (*43, 44*) and some aquaporins are known to be implicated in CO_2 movement (*45–48*) because both proteins act in facilitating the passive CO_2 diffusion process. Our results also demonstrate that decreased photosynthesis after 30 mM fdx treatment is accompanied by respiratory CO_2 (R_l) increasing the CO_2 compensation point (C_i^*) and C_i. Thus, the increase of respiration in the light could be partly responsible for the reduction of P_n after 30 mM fdx treatment. Indeed, the lower rate of CO_2 uptake may result from higher rates of CO_2 loss by respiration in the light. Light response curves indicate that light requirements for photosynthesis is altered after 6 mM fdx. Actually, Γ^*, which reflects the light conditions required for plant photosynthesis and embodies the ability of a plant to utilize high and low light levels, is increased after 6 mM fdx. Therefore, higher Γ^* indicates a higher energy requirement for PSII excitation. In addition, the $\Phi_{PSII}/\Phi CO_2$ ratio, which is an estimate of the relationship between the rate of electron transport and carbon fixation, was approximately 1.5−2-fold higher after fdx treatment, whatever the applied fdx concentration. This implies that more electrons are transported through PSII for each CO_2 molecule assimilated in leaves of treated plants. Therefore, other processes than photosynthesis, such as photorespiration, mitochondrial respiration in the light, N assimilation, and/or

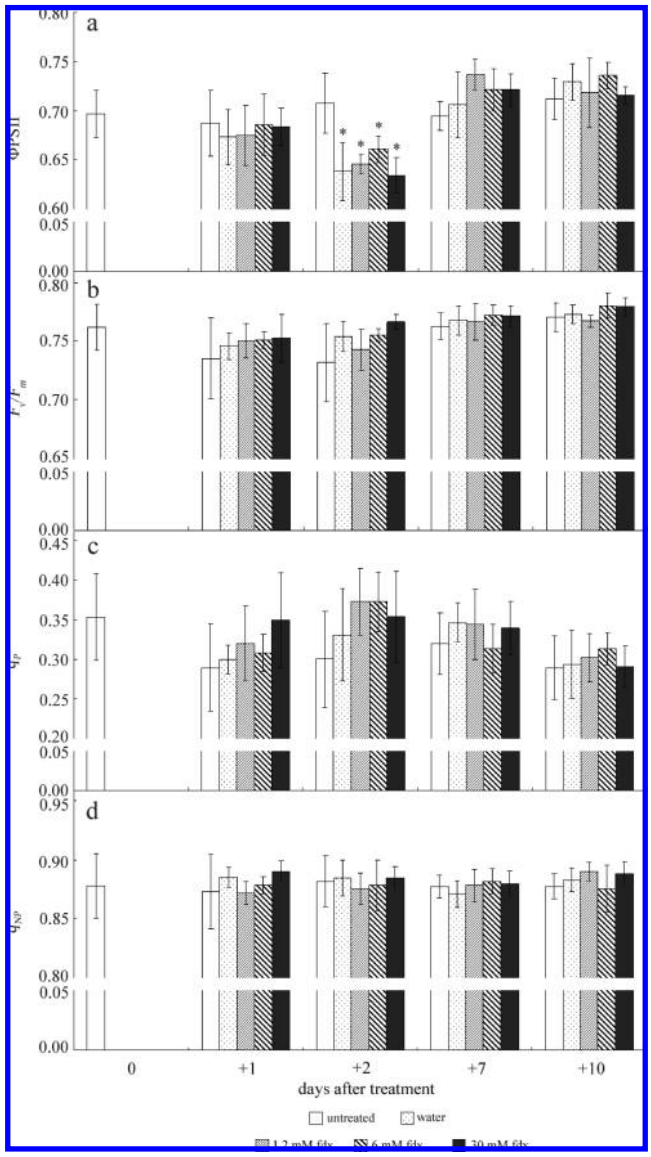

Figure 2. Changes in (**a**) relative quantum yield of PSII (Φ_{PSII}), (**b**) ratio of variable to maximal fluorescence (F_v/F_m), (**c**) photochemical (q_P), and (**d**) total nonphotochemical quenching (q_{NP}) in untreated, water-treated, and fdx-treated leaves of grapevine. Data are means ± standards errors ($n = 24$). Significant differences at $P < 0.05$ between leaves of untreated and treated plants are marked by an asterisk.

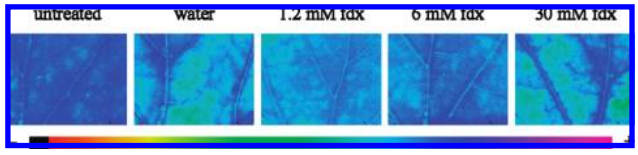

Figure 3. Fluorescence imaging of the abiotic stress induced by fludioxonil. A photograph of relative quantum yield of PSII (Φ_{PSII}) was captured. Data have been mapped to the color palette. The false color code ranges from black (0.000) to pink (1.000), as shown at the bottom of the images.

pseudocyclic electron transport (*22*), may also be operating in fdx-treated leaves. Nevertheless, water application also caused a higher $\Phi_{PSII}/\Phi CO_2$ ratio, indicating that this increase is mostly attributed to the spraying effect.

To locate the possible site of inhibition in the PSII reaction, we also followed DCPIP photoreduction. Oxygen production was inhibited by 6 mM fdx. Nevertheless, since Φ_{PSII} was

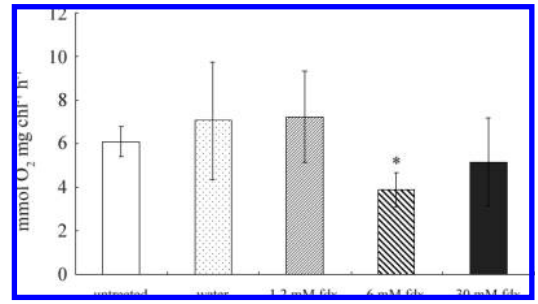

Figure 4. O_2 release by isolated chloroplasts of grapevine leaves from untreated, water-treated, and fdx-treated plants 7 days after fdx application. Data are means ± standards errors ($n = 4$). Significant differences at $P < 0.05$ between leaves of untreated and treated plants are marked by an asterisk.

only affected 2 days after treatment, we can conclude that a decrease in Hill activity does not modify PSII activity.

The recovery of P_n was observed whatever the treatment after 10 days, indicating that fdx did not persistently affect photosynthetic activity. Saladin et al. (*11*) have also noticed that the studied parameters recovered in 10 days for grapevine cuttings treated with fdx. The recovery in P_n may suggest that fdx detoxification occurs in treated leaves.

Each pesticide contains an active ingredient that is responsible for its pesticidal effect. Nevertheless, the active ingredient must be formulated with other nonpesticidal compounds before it is ready to use. In our experiment, fdx was used in a wetting powder in which surfactants can be found. It has been already shown that some surfactants can significantly reduce photosynthesis (*49*). Therefore, the results of this experiment are indicative of the combined phytotoxic effects of both the active ingredient and surfactant.

ABBREVIATIONS USED

Γ*, light compensation point; C_a, carbon dioxide concentration in the air; CCD, charge coupled device; chl, chlorophyll; C_i, intercellular CO_2 concentration; C_i*, CO_2 compensation point; F_0, minimal fluorescence at dark-adapted state; F_0', minimal fluorescence in the light-adapted state; fdx, fludioxonil; F_m, maximal fluorescence in the dark-adapted state; F_m', maximal fluorescence in the light-adapted state; F_t, steady-state fluorescence; F_v/F_m, maximum efficiency of PSII photochemistry after dark adaptation; g_S, stomatal conductance; q_{NP}, nonphotochemical quenching; q_P, photochemical quenching; $Pmax_{(Ci)}$, leaf intercellular CO_2 concentration saturated assimilation rate; $Pmax_{(PPFD)}$, PPFD-saturated CO_2 assimilation rate; P_n, net photosynthetic rate; PPFD, photosynthetic photon flux density; PSII, photosystem II; ΦCO_2, apparent quantum yield of CO_2 fixation; Φ_{PSII}, relative quantum yield of PSII; R_d, dark respiration; R_l, estimation of the mitochondrial respiration in the light; RuBP, ribulose 1,5-bisphosphate; $V_{c,max}$, in vivo maximum rate of rubisco carboxylation.

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