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# Transient Effect of the Herbicide Flumioxazin on Physiology of *Vitis vinifera* L. cv. Pinot Meunier

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## 1. Introduction

Pesticides are widely used to control pests and diseases in crop production. Flumioxazin (fmx), or 2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-indole-1,3(2H)-dione, is a N-phenylphthalimide herbicide registered for pre-emergence control of broadleaved weeds in peanut (*Arachis hypogaea* L.), soybean (*Glycine max* L.), sorghum (Grichar, 2005) and as an early pre-plant burndown treatment in cotton (*Gossypium hirsutum* L.) (Main et al., 2003).

Fmx inhibits protoporphyrinogen oxidase (protox) in the chlorophyll biosynthetic pathway, resulting in light-induced membrane lipid peroxidation (Scott et al., 2001). In the presence of protox inhibitors, tetrapyrroles accumulate, especially protoporphyrin IX (proto IX). Protox inhibition leads to the accumulation of its substrate protoporphyrinogen, which is readily oxidized to proto IX by oxidative enzymes. Proto IX is a quite effective photosensitizer that transfers absorbed light energy to molecular oxygen to form singlet oxygen. The singlet oxygen peroxidizes lipids leading to the destruction of cellular membranes (Moreland, 1999).

Fmx is a pre-emergence herbicide applied on soil at the end of winter at a concentration of 5 mM. Fmx inhibits the development of redroot pigweed (*Amaranthus retroflexus*), lambsquarters (*Chenopodium album*), jimsonweed (*Datura stramonium*), morningglory (*Ipomoea spp.*), nutsedge (*Cyperus spp.*) and prickly sida (*Sida spinosa* L.) (Nagano, 1999; Niekamp et al., 1999).

Fmx enters plants mainly throughout root and tolerant crop species avoid its injury by rapid detoxication metabolism (Yoshida et al., 1991). Fmx is applied to control adventive plants but the presence of such molecules in the foliage of non-target crops and in the soil has been reported (Jame et al., 1999). Little information is available on the effect of fmx on crop physiology, especially in grapevine, although it is one of the most frequently used herbicides in vineyards. We showed that fmx dramatically affects grapevine physiology in

*vitro* (Saladin et al., 2003a, b, c, d). Various concentrations of this herbicide have a negative impact on vine plantlet leaf growth, as revealed by tissue dehydration and cell membrane alteration, decrease in osmotic potential and accumulation of proline. Moreover, fmx treatment results in a reduction of plantlet growth and photosynthesis and further induces some perturbation in leaf carbohydrate partitioning (Saladin et al., 2003b). Proteomic analysis of grapevine under fmx stress suggested that photosynthesis-related proteins, enzymes involved in photorespiration and enzymes of sugar metabolism were impaired (Castro et al., 2005). However, these results were obtained with juvenile plantlets grown *in vitro* and thus have to be considered cautiously before being extended to the whole plant cultivated in vineyards.

The aim of this study is to further determine the effects of fmx treatment on the photosynthetic characteristics of grapevine cutting leaves. The combined measurements of chlorophyll fluorescence and gas-exchange rates were proved to be a useful approach for distinguishing stomatal *versus* nonstomatal effects, as well as for estimating the importance of various types of energy use, such as thermal dissipation and photorespiration (Hendrickson et al., 2004).

## 2. Materials and methods

### 2.1 Plant material, growth conditions

Canes of *Vitis vinifera* L. cv. Pinot Meunier were collected in winter, treated with cryptonol (2% v/v) to prevent contamination and stored in the dark at 4 °C for a minimum of two weeks. They were then cut into fragments to obtain two consecutive fertile buds and one sterile bud (Mullins, 1966; Mullins & Rajasekaran, 1981). The sterile bud was soaked for three min in a 0.1% (w/v) 3-indol butyric acid aqueous solution in order to stimulate rhizogenesis. Then, the cuttings were placed in 300 ml pots containing perlite: sand (1: 2) at 25 °C and 75% relative humidity in the greenhouse, with a 16 h photoperiod at a photosynthetic photon flux density of 400  $\mu\text{mol m}^{-2}\text{s}^{-1}$  (Lebon et al., 2005).

### 2.2 Fmx treatments

Plants were daily irrigated with a nutrient solution optimized for grapevine culture (Coïc & Lesaint, 1971). After eight weeks, when the cuttings had eight leaves, the fmx solution (commercial herbicide Pledge®) was sprayed only one time on the soil with different solutions of fmx in water: 0.5 mM, 5 mM (concentration recommended by the manufacturer) or 50 mM. Simultaneously, the soil of control cuttings was sprayed with water.

### 2.3 Growth measurements

At the end of the experimentation, ten plants per treatment were harvested, separated into shoot and root parts, and their fresh weights were determined.

### 2.4 Measure of gas exchanges

The net photosynthetic rate ( $P_n$ ), the stomatal conductance ( $g_s$ ), the intercellular  $\text{CO}_2$  concentration ( $C_i$ ) and the transpiration rate ( $T$ ) were measured using a portable infrared gas analyser (LI-Cor Model 6400, Lincoln, NE, USA). The infrared gas analysis system was equipped with a clamp-on leaf cuvette that exposed 6  $\text{cm}^2$  of leaf area. Light, temperature and humidity were 400  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ,  $25 \pm 1$  °C and 30% respectively.

**Photosynthetic light response curves:** Response of  $P_n$  to photosynthetic photon flux (PPF) was measured by illuminating the leaf at decreasing PPF (from 2000 to 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) until  $P_n$  was constant. The apparent quantum yield of  $\text{CO}_2$  fixation ( $\Phi\text{CO}_2$ ) was calculated as the slope of the linear portion of the response curves between 0 and 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF.  $\text{CO}_2$  was maintained at a constant level of 360  $\text{mmol l}^{-1}$  using an LI-6400-01  $\text{CO}_2$  injector (LI-Cor 6400 Lincoln, NE, USA) with a high pressure liquefied  $\text{CO}_2$  cartridge source.

## 2.5 Chlorophyll fluorescence measurements

The chlorophyll *a* fluorescence of the leaves was quantified on attached leaves with an IMAGING-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany). The measuring system applies array of blue light-emitting diodes (LEDs) (peak wavelength, 470 nm) for saturating light pulses. The frequency of the pulses was adjusted to 10 Hz. Measurements were carried out at maximal distance between the camera and the leaf, corresponding to a 25 x 34 mm area. The image captured by the charge-coupled device (CCD) camera was composed of 640 x 480 pixels.

During the whole experiment, the measurements were systematically performed on the adaxial side on the central parts of the young leaves. The leaves used for measurements were pre-conditioned in the dark. The initial fluorescence ( $F_o$ ) was obtained after 0.5 hour of dark adaptation. Maximal fluorescence ( $F_m$ ) was obtained with a saturating flash (1 s, 13 000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The ratio of variable to maximal fluorescence ( $F_v/F_m$ ) was calculated. The protocol for fluorescence measurement was similar to the one described by Genty *et al.* (1989), but the measurements were performed on attached leaves. The relative quantum yield of PSII ( $\Phi_{\text{PSII}}$ ) at steady state is defined as  $(F'_m - F_s)/F'_m$  where  $F_s$  and  $F'_m$  are respectively steady-state fluorescence and maximum fluorescence in the light.  $\Phi_{\text{PSII}}$  represents the number of electrons transported by a PSII reaction centre per mole of quanta absorbed by PSII. Both Photochemical ( $q_p$ ) and total non-photochemical quenching ( $q_{\text{NP}}$ ) were calculated according to van Kooten & Snel (1990). The Stern-Volmer equation (NPQ) was used as an indicator of the activity of energy dissipation in the pigment bed of PSII. NPQ was proportional to the effective rate constant for energy dissipation in the antennae as well as in the concentration of quenching centres (Demmig-Adams *et al.*, 1996).

**Fluorescence light response curves:** Response of  $F_v/F_m$ ,  $Q_P$  and  $Q_{\text{NP}}$  to PPF (the light response curve) were measured by illuminating the leaf with actinic light at increasing PPF (0 to 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

## 2.6 Chlorophyll assay

Chlorophyll contents were determined at the end of the experiment. Leaf slices were dissected and pigments were extracted under overnight continuous agitation in 80% (v/v) acetone amended with 0.5% (w/v)  $\text{MgCO}_3$  to prevent chlorophyll acidification at 4°C. Crude extract was centrifuged at 10,000 g for 10 min at 4°C, and the supernatant was used to estimate spectrophotometrically pigment concentrations according to the absorbance coefficients determined by Lichtenthaler (1987). Results were expressed in  $\text{mg g}^{-1}$  fresh weight (FW).

## 2.7 Statistical analysis

Five replicates plants per treatment and three replicate measurements per plant were carried out. All data were analysed using the Mann & Whitney test at the 0.05 probability level.

### 3. Results

#### 3.1 Growth

In the event of fmx excess (5 and 50 mM), the plant growth is inhibited (Fig. 1). The leaves growth is more affected than the root growth.

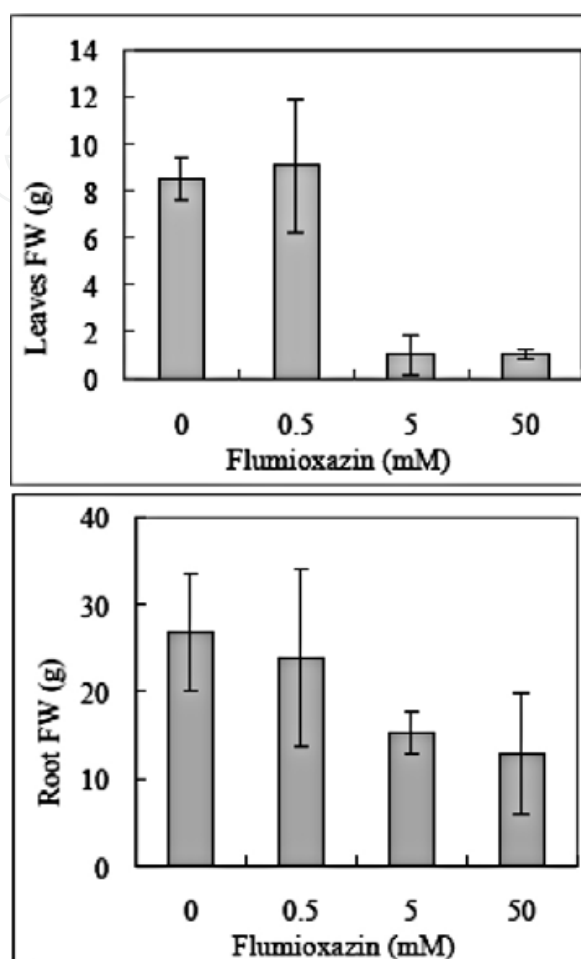


Fig. 1. Leaves and root fresh weight of grapevine after the spraying of various fmx concentrations. Each value is the mean of 15 measurements  $\pm$  SD.

#### 3.2 Gas exchanges

Photosynthetic responses of grapevine grown with various herbicide concentrations were analysed to determine whether fmx modifies  $P_n$ ,  $g_s$ ,  $T$  and  $C_i$ . The  $P_n$  decreased significantly after one day at 0.5, 5 and 50 mM fmx (Fig. 2A). Fifteen days after spraying with 5 and 50 mM fmx,  $P_n$  was steady and equal to zero.  $g_s$  was significantly affected by fmx (Fig. 2B). Detectable inhibition of  $g_s$  occurred after one day using 5 and 50 mM fmx and after three days at 0.5 mM fmx. Whatever fmx concentration, the treatment leads to the closure of stomata in grapevine after three days and 15 days after,  $g_s$  was equal to 0 for 5 and 50 mM. Transpiration of grapevine following fmx treatment was also reduced significantly at 0.5, 5 and 50 mM after two days (Fig. 2D).  $C_i$  was not affected with 0.5 mM fmx, but  $C_i$  increased at 50 mM after 3 days and at 5 mM fmx after 5 days (Fig. 2C). At 0.5 mM fmx and after 5 days,  $P_n$  and  $T$  increased and after 45 days, they were equal to control.

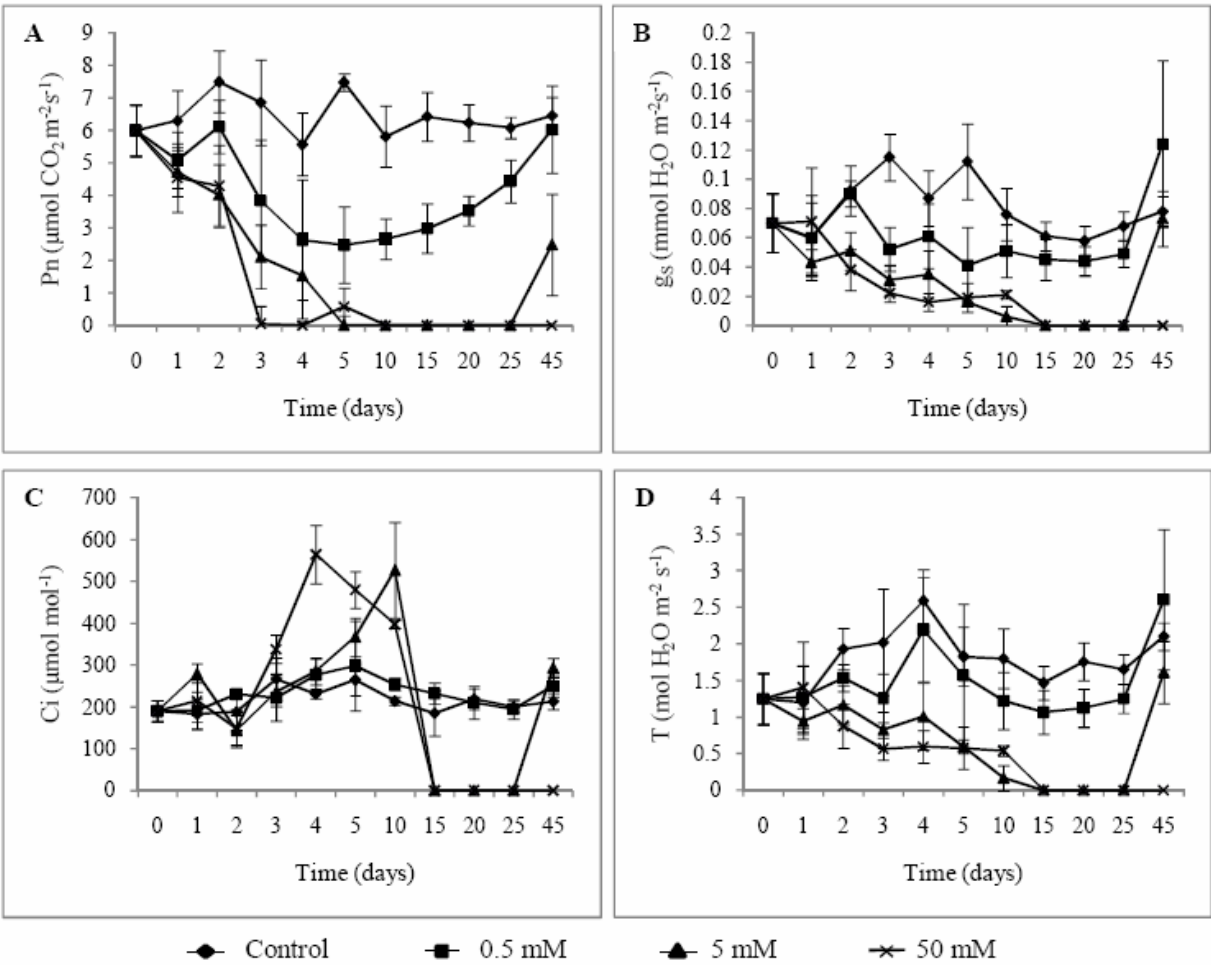


Fig. 2. Changes in net photosynthesis ( $P_n$ ) (A), stomatal conductance ( $g_s$ ) (B), intercellular  $\text{CO}_2$  concentration ( $C_i$ ) (C), and transpiration rate ( $T$ ) (D) in leaves of grapevine after the spraying of various fmx concentrations. Each value is the mean of 15 measurements  $\pm$  SD.

*Photosynthetic light response curves*

To clarify the nature of the mechanisms involved in plant adaptation to the treatment, both  $\text{CO}_2$  distribution in the leaf and the capacities of mesophyll to assimilate atmospheric  $\text{CO}_2$  were analyzed after ten days. The study of these processes was performed by analysing the light response curve and by measuring  $P_n$  variations in response to the increase of  $\text{CO}_2$  concentration after ten days of herbicide stress application. The leaves of the treated plants showed a drastically lower photosynthetic capacity than the control leaves. Similarly, saturating PPF and apparent quantum yield of  $\text{CO}_2$  fixation ( $\Phi\text{CO}_2$ ) showed a significant decrease with increasing fmx concentrations (Table 1). Cuttings treated with 50 mM fmx did not respond to the PPF because the plants died. Also, dark respiration and compensation point decreased when the fmx stress increased. In addition, photosynthesis was saturated at  $10.86 \mu\text{mol m}^{-2} \text{ s}^{-1}$  in the control and light-saturated net  $\text{CO}_2$  assimilation rate ( $A_{\text{sat}}$ ) decreased by 15% and 98% using 0.5 and 5 mM fmx respectively. Using the high PPF, the slope of curves was null (Table 1), meaning that there was no photoinhibition. The ratio  $\Phi\text{PSII}/\Phi\text{CO}_2$  was inversely correlated to the efficiency of light involvement for carbon fixation. It was higher in leaves grown at 5 mM fmx concentration (Table 1), indicating that the light was less efficient in treated cuttings.



	Fmx concentrations			
	Control	0.5 mM	5 mM	50 mM
Apparent quantum yield of CO <sub>2</sub> fixation (ΦCO <sub>2</sub> )	0.044±0.002 <sup>a</sup>	0.041±0.006 <sup>a</sup>	0.006±0.007 <sup>b</sup>	-
Dark respiration Rd (μmol m <sup>-2</sup> s <sup>-1</sup> )	1.10±0.54 <sup>a</sup>	1.31±0.34 <sup>a</sup>	0.622±0.710 <sup>b</sup>	-
Compensation point Γ (μmol)	26.32±1.30 <sup>a</sup>	31.67±3.01 <sup>b</sup>	101.42±10.01 <sup>c</sup>	-
A <sub>sat</sub> (μmol m <sup>-2</sup> s <sup>-1</sup> )	10.86±1.28 <sup>a</sup>	9.14±0.93 <sup>b</sup>	0.139±0.055 <sup>c</sup>	-
PPF (μmol m <sup>-2</sup> s <sup>-1</sup> ) for A <sub>sat</sub>	1000 <sup>a</sup>	500 <sup>b</sup>	250 <sup>c</sup>	-
Slope with PPF > 1500 μmol m <sup>-2</sup> s <sup>-1</sup>	-0.0003±0.0008 <sup>a</sup>	0.0003±0.0001 <sup>a</sup>	0.0005±0.0003 <sup>a</sup>	-
ΦPSII/ΦCO <sub>2</sub>	15.43 <sup>a</sup>	12.92 <sup>b</sup>	50.33 <sup>c</sup>	-

Table 1. Analyses of photosynthetic light response curves: the apparent quantum yield of CO<sub>2</sub> fixation (ΦCO<sub>2</sub>), dark respiration (Rd), compensation point (Γ), light-saturated net CO<sub>2</sub> assimilation rate (A<sub>sat</sub>), PPF (μmol m<sup>-2</sup> s<sup>-1</sup>) for A<sub>sat</sub>, slope with PPF > 1500 μmol m<sup>-2</sup> s<sup>-1</sup> and ratio ΦPSII/ΦCO<sub>2</sub> of grapevine with various flumioxazin concentrations after ten days. The grapevine treated with 50 mM of fmx were dead. Statistical analyses were carried out using the Mann and Whitney test. Means for a considered parameter were not significantly different when followed by the same letter (P ≥ 0.05).

3.3 Chlorophyll fluorescence

All chlorophyll fluorescence parameters strongly dropped as the fmx concentration increased (Fig. 3). The Fv/Fm ratio used as the means of maximal photochemical efficiency of PSII was not modified in the controls nor in the 0.5 mM fmx treated cuttings, whereas it dropped down to zero after four days using 5 and 50 mM fmx (Fig. 3A). Similarly ΦPSII slowed down significantly after four days of 5 and 50 mM fmx treatments (Fig 3B). Quenching was also affected in the same way: q<sub>P</sub> and q<sub>NP</sub> decreased significantly after ten days using 5 and 50 mM fmx (Fig. 3C, D). There was no PSII activity after 10 days using 50 mM fmx and after 15 days using 5 mM.

Identification of fmx damage in the leaf

Picture of the fluorescence showed a marked decrease in fluorescence emission when the cuttings were exposed to the highest concentrations of fmx (Fig. 4). Fm images allowed early detection of fluorescence variations than Fv/Fm images. 0.5 mM fmx treatment induced only a slight fluorescence decline in the leaves during the whole treatment (Fig. 3). More drastic modifications appeared in the veins of leaves from four days using 5 mM fmx (Fig. 4). Using 50 mM fmx, the fluorescence decline appeared significant after four days in the veins and next spread rapidly throughout the entire leaf, the damages spread throughout the mesophyll (Fig. 4).

Fluorescence light response curves

Figure 5 presents the changes in light response curves of chlorophyll fluorescence in leaves ten days after fmx treatment. The responses of Fv/Fm, Q<sub>P</sub> and Q<sub>NP</sub> to PPF were measured by illuminating the leaf with actinic light at increasing PPF 0 to 1200 μmol m<sup>-2</sup> s<sup>-1</sup>. Treated plants responded less strongly to the light than the control. Fv/Fm and Q<sub>P</sub> decreased with

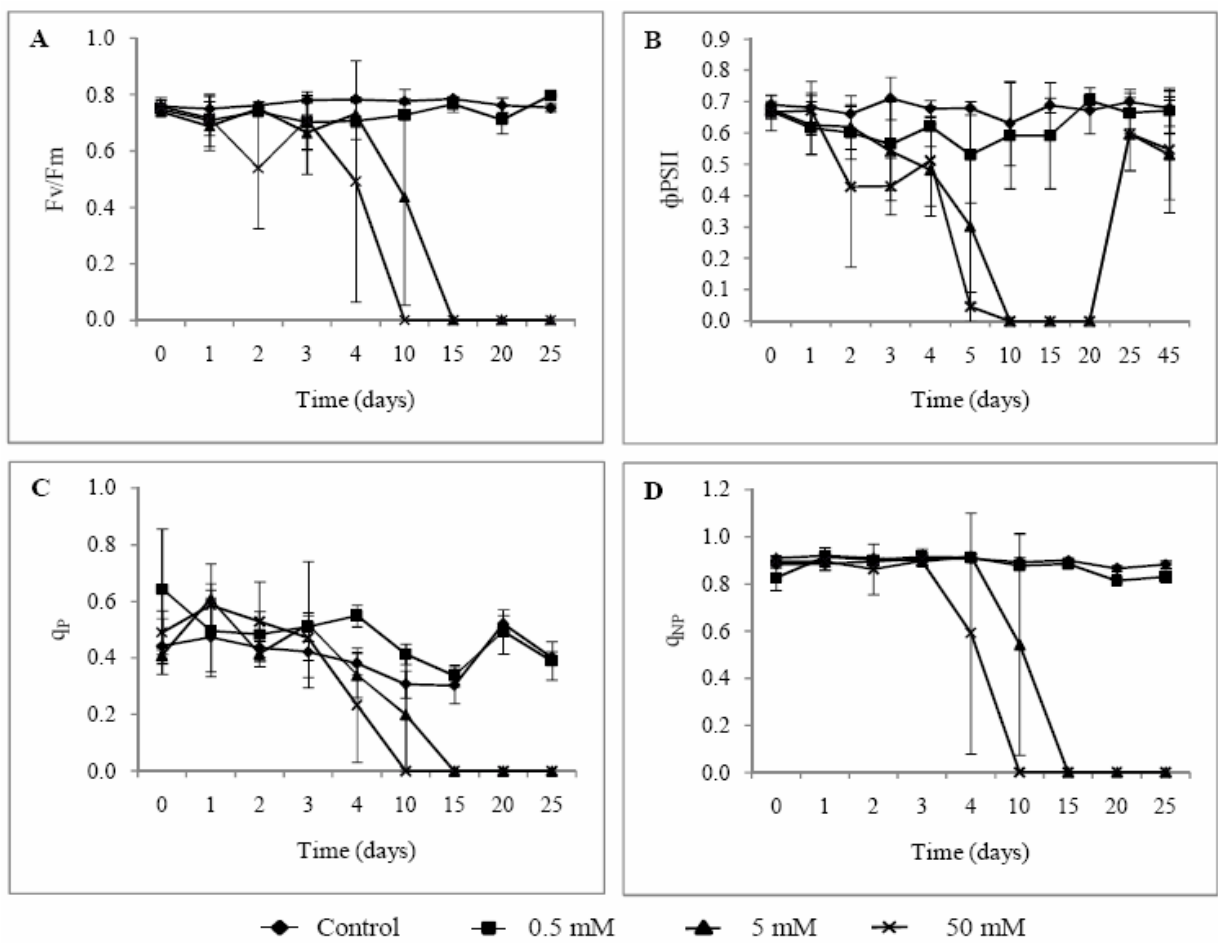


Fig. 3. The Chl fluorescence parameters of PSII (relative units) in leaves of grapevine after the spraying of various fmx concentrations. Each value is the mean of 15 measurements  $\pm$  SD.

increasing light intensity while  $Q_{NP}$  increased. The fluorescence kinetics showed that the increase of fmx concentration led to a decrease in the maximal efficiency of PSII photochemistry and a decrease in the coefficients of photochemical and non-photochemical quenchings.

### 3.4 Chlorophyll contents

Fmx leads a decrease in the total chlorophyll, chlorophyll a and b concentration and in the carotenoid concentration. We measured a decline in the ratio chlorophyll a/chlorophyll b (Table 2).

	Fmx concentration			
	Control	0.5 mM	5 mM	50 mM
Chl tot (mg.g FW)	1.728 $\pm$ 0.259	0.995 $\pm$ 0.302	0.593 $\pm$ 0.198	0.021 $\pm$ 0.016
Chl a (mg.g FW)	1.368 $\pm$ 0.168	0.749 $\pm$ 0.211	0.464 $\pm$ 0.136	0.018 $\pm$ 0.014
Chl b (mg.g FW)	0.359 $\pm$ 0.097	0.245 $\pm$ 0.112	0.128 $\pm$ 0.076	0.002 $\pm$ 0.008
Carot (mg.g FW)	0.628 $\pm$ 0.057	0.348 $\pm$ 0.083	0.263 $\pm$ 0.055	0.037 $\pm$ 0.006
Chl a / Chl b	3.927 $\pm$ 0.594	3.378 $\pm$ 0.976	2.972 $\pm$ 0.644	0

Table 2. Chlorophyll total, a, b and carotenoid concentration and chlorophyll a / chlorophyll b ratio with various fmx concentrations. Each value is the mean of 15 measurements  $\pm$  SD.



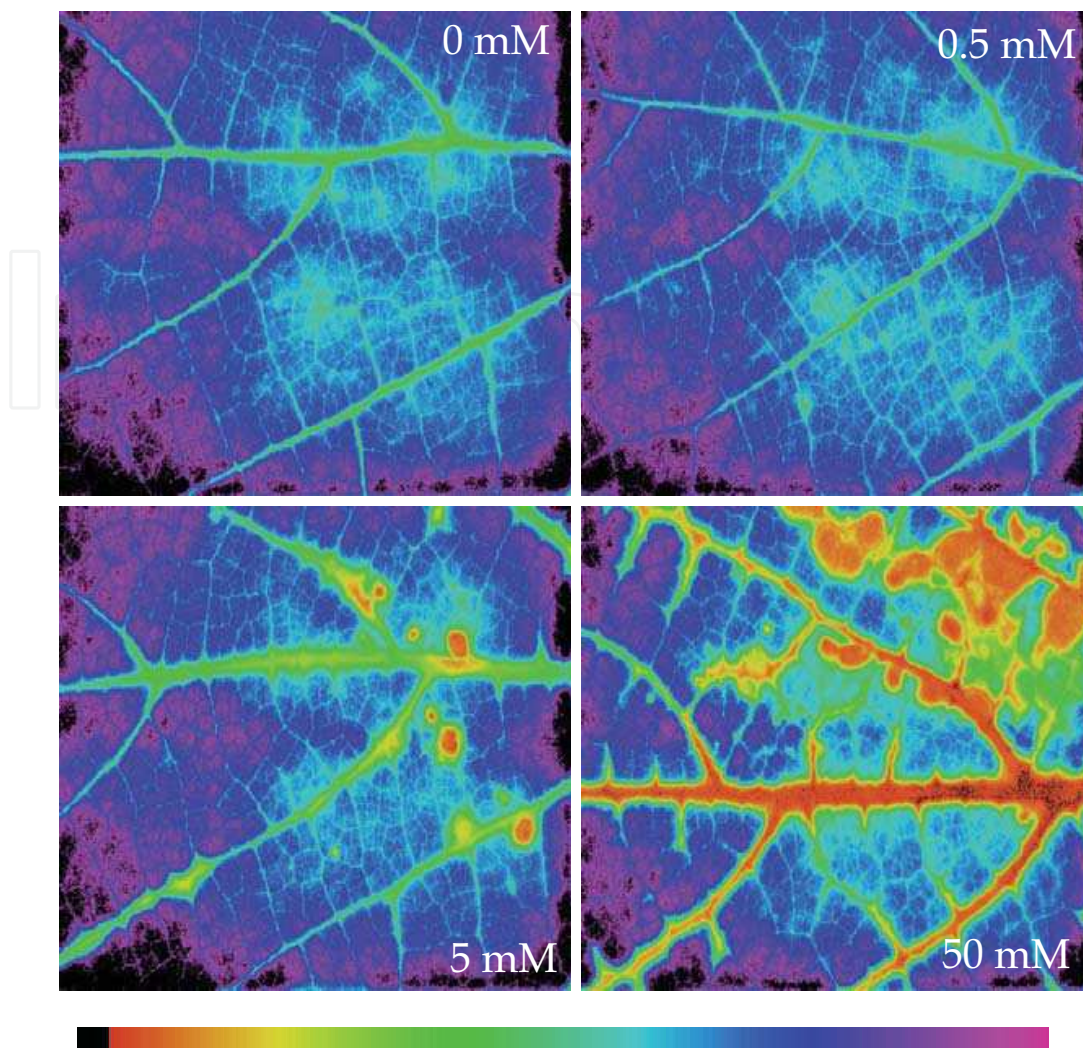


Fig. 4. Fluorescence imaging of the dynamic evolution of abiotic stress induced 4 days after flumioxazin treatment. A grapevine leaf was 30 min dark-adapted and submitted to saturation pulse. A photograph of maximum fluorescence ( $F_m$ ) was captured. Data have been mapped to the colour palette. The false colour code ranges from black (0.000) to pink (1.000), as shown at the bottom of the images.

#### 4. Discussion

These results provide new insights into the effects of fmx herbicide on grapevine physiology through the analysis of numerous parameters. We have demonstrated a transient fmx effect on Pinot Meunier physiology. The answer of this cultivar was different that observed with the Chardonnay (Bigot et al, 2007). They complement preliminary information on the stress effects of this herbicide on plant physiology *in vitro* (Saladin et al., 2003a, b, c, d; Castro et al., 2005) and help to further understand how action of herbicide acts on non-target grapevine. The soil-applied herbicide is known to be a peroxidizing agent, through the inhibition of protoporphyrinogen IX oxidase in the chlorophyll biosynthetic pathway (Scott et al., 2001). It appears that fmx affects other metabolic functions i.e. all the photosynthetic parameters we evaluated. It induces a strong net photosynthesis inhibition and a parallel decrease of stomatal conductance and transpiration. The photosystem II activity is also affected.

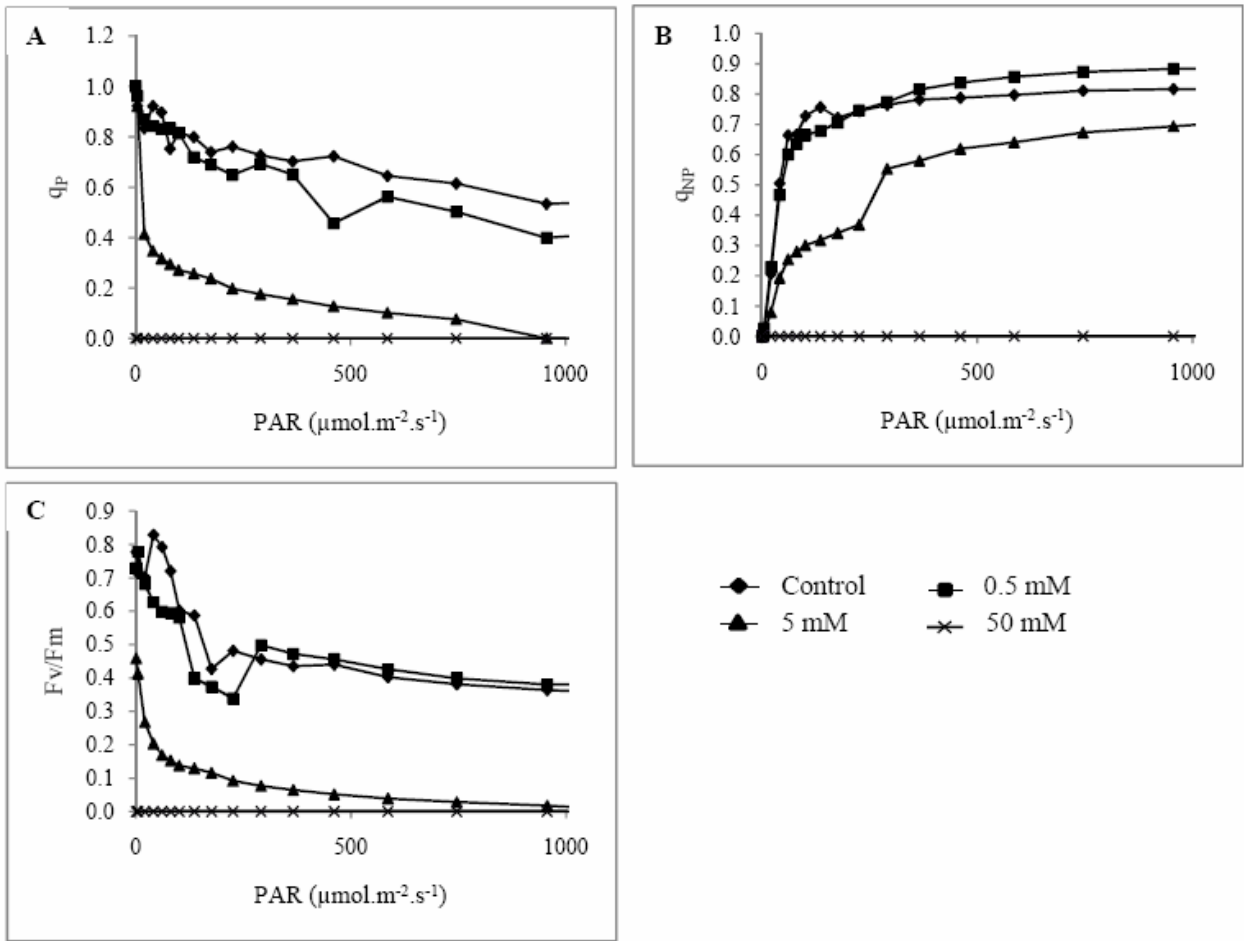


Fig. 5. Fluorescence light response curves:  $Q_p$  (A),  $Q_{NP}$  (B),  $F_v/F_m$  (C), to PPF (0 to 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in leaves of grapevine after ten days with various fmx concentrations.

**Fmx inhibits  $\text{CO}_2$  assimilation**

All the photosynthetic parameters of grapevine cutting leaves were significantly reduced after 25 days of fmx treatment. Net photosynthesis and transpiration reduction were associated with decline of stomatal conductance. The photosynthesis decrease in leaves may be caused by stomatal closure. However, the reduction of  $F_v/F_m$  and the quantum yield of  $\text{CO}_2$  assimilation indicate that the efficiency of photochemistry is also impaired in grapevine treated with 5 and 50 mM fmx.

There is a strong relationship between photosynthetic electron transport and carbon fixed by plants (Genty et al., 1989).  $\Phi\text{PSII}/\Phi\text{CO}_2$  is an estimate of the relationship between the rate of electron transport and carbon fixation. If four electrons are consumed per mol  $\text{CO}_2$  fixed and if the light is equally distributed between the two photosystems,  $\Phi\text{PSII}/\Phi\text{CO}_2$  should theoretically be 8 minimum. Experimentally,  $\Phi\text{PSII}/\Phi\text{CO}_2$  greater than 8 is obtained, meaning that electrons are also used for other processes than photosynthesis, such as photorespiration, N assimilation, or pseudocyclic electron transport (Genty et al., 1989).

**Fmx affects chlorophyll a fluorescence**

The fluorescence arising from chlorophyll is almost exclusively associated with PSII (Schreiber et al., 1994). Since PSII functioning is sensitive to a wide range of environmental

variations, chlorophyll fluorescence provides numerous information on the effects of stresses on plants (Schreiber et al., 1994). Our results clearly show that fmx significantly inhibits the quantum yield of PSII electron transport ( $\Phi_{PSII}$ ) in grapevine cuttings. We also demonstrate that such a decrease in  $\Phi_{PSII}$  was associated to the alteration of  $q_P$  and  $q_{NP}$ . A decrease in  $q_P$  induced by fmx indicates a higher proportion of closed PSII reaction centres, i.e., an increase in the proportion of the reduced state of  $Q_A$ , (Genty et al., 1989), which probably generates a decrease in the proportion of available excitation energy used for photochemistry (Havaux et al., 1991). Concomitant with the reduction of  $F_v/F_m$ , we observed that  $q_{NP}$  decreased drastically with increasing fmx concentrations, suggesting that the fmx treatment does not involve non-radiative energy dissipation.

In leaves of grapevine cuttings, the capacity for  $CO_2$  assimilation decreases to almost zero after five days whatever fmx concentration. However, after ten days of 0.5 mM fmx treatment, while there is negligible  $CO_2$  assimilatory capacity,  $\Phi_{PSII}$  remains at approximately 12% when compared to control leaves. This suggests that a certain rate of non-cyclic electron transport is required to maintain  $CO_2$  assimilation. An alternative way to  $CO_2$  assimilation for electrons would be oxygen reduction by photorespiration and/or a Mehler reaction (Brestic et al., 1995). Changes in fluorescence yield in grapevine leaves are also associated with modifications in the antenna pigments, in the efficiency of excitation trapping at the active centres of PSII, or in changes in the thylakoid membrane (Calatayud & Barreno, 2001). The *in vitro* application of fmx to grapevine induced, on the one hand, disorganization of internal photosynthetic membranes (Saladin et al., 2003a) and affected, on the other hand, an oxygen-evolving enhancer protein and a LHCII type III chlorophyll a/b binding protein, a major component of light-harvesting antennae complex of PSII (Castro et al., 2005).

Fmx treatment on the soil, provokes leaf fluorescence damages that first occur in the veins and next spread throughout the mesophyll. Fmx treatment induces depression of photosynthesis in grapevine and involves also heterogeneity of leaf photosynthesis. Such heterogeneity may be the consequence of patchy stomatal closure and/or collapse of part of the mesophyll due to loss of turgor, associated with a low lateral  $CO_2$  diffusion capacity (Cornic & Massacci, 1996). It also results in decreases in the photosynthetic efficiency and capacity of leaves. These observations further suggest that either fmx or a by-product penetrate the plant throughout the roots and are thus distributed in the whole plant through the veins. These results are consistent with Castro et al. (2005) who found significant changes in root and shoot proteome and who suggest that the herbicide could act systemically in grapevine tissues, probably *via* root uptake.

## 5. Conclusion

We have demonstrated a transient fmx effect on grapevine physiology characterized by strong increases of  $P_n$ ,  $g_s$  and  $\Phi_{PSII}$  at 0.5 and 5 mM fmx after 45 days. The grapevine was able to partially overcome the damages caused by herbicides (Saladin & Clément, 2005). In the vineyard the herbicide caused mild stress (Saladin et al., 2003c, d). It may be explained by a detoxification of the herbicide in the rootstock and/or a low fmx uptake by the roots, which is due to a deeper root system or different soil adsorption characteristics (Saladin et al., 2003d). Moreover, in the vineyard fmx was applied at the end of the winter, when canes have no leaf and when the sap flow is low.



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## **Herbicides and Environment**

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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