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REGULAR PAPER

Influence of the acetolactate synthase inhibitor metsulfuron-methyl on the operation, regulation and organisation of photosynthesis in *Solanum nigrum*

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Abstract The influence of the acetolactate synthase inhibitor metsulfuron-methyl on the operation of the photosynthetic apparatus was examined on 4-weeks-old climate chamber-grown *Solanum nigrum* plant. To have an indication on the relative performance of the photosynthetic apparatus of ALS-treated plants, the level of carbon dioxide (CO_2) fixation, the relative quantum efficiency of photosystem I (Φ_{PSI}) or photosystem II (Φ_{PSII}) electron transport and leaf chlorophyll content were assessed for both control and treated plants at 2, 4 and 7 days after application of the herbicide. Results indicated a progressive inhibition of the level of CO_2 fixation, the relative quantum efficiency of photosystem I (Φ_{PSI}) and II (Φ_{PSII}) electron transport and the leaf chlorophyll content already 2 days after application of the herbicide. The linear relationship between the photosystem I and II was unaltered by herbicidal treatment and was sustained under conditions where large changes in pigment composition of the leaves occurred. It appears that the stress-induced loss of leaf chlorophyll is not a catastrophic process but rather is the consequence of a well-organised breakdown of components. Under photorespiratory and non-photorespiratory conditions, the relationship between the index of electron transport flow through photosystem I and II and the rate of CO_2 fixation is altered so that electron transport becomes less efficient at driving CO_2 fixation.

Keywords Herbicide · Photosystem I · Photosystem II · Quantum efficiency · Sulfonylurea · CO_2 fixation · Chlorophyll · Chlorophyll fluorescence

Abbreviations

ALS	acetolactate synthase
DAT	days after treatment
HRAC	herbicide resistance action committee
Φ_{PSI}	relative quantum efficiency of photosystem I
Φ_{PSII}	relative quantum efficiency of photosystem II

Introduction

The acetolactate synthase (ALS) inhibitor herbicides (Herbicide Resistance Action Committee group B), in which the sulfonylureas are a particular sub-group, selectively inhibit acetolactate synthase (EC 4.1.3.18), which is the first enzyme involved in chloroplastidic biosynthesis of essential branched-chain amino acids. Although visual symptoms, including anthocyanin accumulation (Suttle and Schreiner 1982), leaf chlorosis and necrosis of the growing points (Blair and Martin 1988) may not appear until several days to weeks after herbicide application, profound changes take place shortly after application of ALS herbicides.

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Metabolic and physiological responses to sulfonylureas include a rapid cessation of growth that occurs within hours after the application (Gaston et al. 2003; Rhodes et al. 1987; Shaner and Singh 1991), the inhibition of the mitosis (Ray 1982; Rost and Reynolds 1985) and DNA synthesis (Ray 1982), the increase in free amino acids (Rhodes et al. 1987), the rapid decrease in the level of soluble proteins (Shaner 1989) and a decrease in the translocation of photosynthate to the growing points of the plant (Devine 1989 cited in Shaner and Singh 1992). See Shaner (1989) for review. It has been suggested by Shaner and Singh (1992) that all these factors probably interact to kill the plants.

Photosynthesis is not regarded to be a primary target of ALS-inhibiting herbicides, but changes in chlorophyll fluorescence responses have already been observed in treated plants. Judy et al. (1990) found effects on the fluorescence from barley 2 h after treatment with imazaquin, and Percival and Baker (1991) found effects on the fluorescence from wheat leaves 24 h after treatment with the ALS-inhibitor imazamethabenz-methyl at the recommended field rates. Being able to detect early effects on photosynthesis of herbicides whose mode of action does not directly affect photosynthesis also has practical implications. The Minimum Lethal Herbicide Dose (MLHD) technology (Kempenaar et al. 2002; Ketel 1996) is a decision-support system leading to the use of lower rates of photosynthesis-inhibiting herbicides. The method allows the calculation of the minimum dose of herbicide needed to control a weed population and provide an early detection tool based on simple and rapid measurements of photosystem II activity to evaluate the efficacy of the treatment. Extension of the MLHD technology to the acetolactate synthase inhibitors herbicides requires the identification of suitable parameters for evaluation of the activity of the herbicides shortly after application. This last element is of particular importance for ensuring that even though minimal doses of herbicides have been employed, there is a guarantee that the treatment will be successful in eliminating the weeds. Such a guarantee has contributed to the adoption of the MLHD methodology (Kempenaar et al. 2004).

The response of the photosynthetic system to stress involves changes at the metabolic and electron transport level; stress results in decreases in the rate of CO_2 fixation, and chloroplast electron transport monitored by means of measurements of the quantum efficiencies for electron transport by photosystems I and II (De Groot et al. 2003; Kingston-Smith et al. 1997, 1999). The individual responses of these processes are interesting in their own right, but in addition by comparing these processes with each other it is possible to understand more of how they regulate in response to stress and what the limits of this response are (De Groot et al. 2003; Harbinson et al. 1990a; Kingston-Smith et al. 1997, 1999; Laisk and Oja 1994). This work has so far shown that in the short-term regulation of electron transport is achieved by changing the capacity of the electron transport chain between photosystems I and II probably by adjusting intrathylakoid pH (Genty and Harbinson 1996; Laisk and Oja 1994); it is not known to what extent this mechanism operates during long-term stress. It is clear, however, that in long-term stress the overall co-ordination of the electron transport chain, and its relationship with photosynthetic metabolism is remarkably stable (De Groot et al. 2003; Kingston-Smith et al. 1997, 1999).

The aim of the study was to see whether sulfonylurea herbicides had an effect, even though indirect, on the operation of the photosynthetic apparatus of *S. nigrum* plants. Special emphasis was put on how these effects on photosynthesis developed in time. Another objective of the work was to determine how the regulation and protection of the photosynthetic apparatus operates under conditions of prolonged and severe metabolic dysfunction provoked by the hydrolysis of soluble proteins. Finally, this work should give more understanding on the possible processes of morbidity related to the type of herbicide used and the cessation of growth.

Material and methods

Plant material and spraying procedure

Seeds of *Solanum nigrum* (black nightshade) were germinated on a moist mixture of potting soil and sand (2:1). Fifteen days after sowing individual plants were transferred into 1-dm^3 pots and placed in a growth chamber with a photosynthetically active radiation (PAR) of 220 μmol m^{-2} s^{-1} for 12 h (lamps: TL-D-HF, Philips, Eindhoven, The Netherlands). The plants were grown at day/night temperatures of 22°C/18°C and 70% relative humidity. Water and soil nutrients were supplied so as not to be limiting for growth.

Four-week-old (3-leaf stage) *S. nigrum* plants were sprayed with an air-pressurised laboratory track sprayer delivering 400 l ha^{-1} herbicide solution at 303 kPa. *S. nigrum* plants were treated with 16 g a.i. ha^{-1} of metsulfuron-methyl (Ally, 40 g l^{-1}, DuPont) and 0.75% v/v isodecyl ethoxylate (Trend 90, DuPont). Isodecyl ethoxylate was used as a surfactant to improve the penetration and uptake of metsulfuron-methyl by the leaves.

Photosynthesis measurements

To have an indication on the relative performance of the photosynthetic apparatus of ALS-treated plants the level of carbon dioxide (CO_2) fixation, the relative quantum efficiency of photosystem II electron transport (Φ_{PSII}) and the relative quantum efficiency of photosystem I electron transport (Φ_{PSI}) were assessed for both control and treated plants. Equipment similar to that described by De Groot et al. (2003) was used. The CO_2 fixation was measured using an Infra-Red gas analyser (Mark 3, Analytical Development Company, Hoddesdon UK). Actinic light was provided by a quartz halogen lamp filtered by Near Infra Red (NIR) and Calflex dichroic mirrors (Balzers, Liechtenstein), and light-intensity was adjusted using metal-film neutral density filters (Balzers, Liechtenstein) (De Groot et al. 2003). Two excitation wavelengths, 560 and 660 nm, were used to excite the chlorophyll fluorescence in order to measure Φ_{PSII}; the fluorescence they each produce was electronically recovered and displayed separately. The efficiencies derived from the two excitation wavelengths will be referred to as $\Phi_{PSII560nm}$ and $\Phi_{PSII660nm}$, respectively. The Φ_{PSI} was measured using the irradiance-induced absorbance change around 820 nm (Harbinson and Woodward 1987). The $\Phi_{PSII560nm}$ is used in correlations with Φ_{PSI} and the $\Phi_{PSII660nm}$ is used in correlations with CO_2 fixation (Kingston-Smith et al. 1997). The 820-nm absorbance changes used to measure Φ_{PSI} efficiency also permitted the measurement of the kinetics of electron transport between the plastoquinol pool and P-700$^+$ by measuring the reduction of the photochemically formed P-700$^+$ pool following a light-on light-off transition (Harbinson and Hedley 1989; Laisk and Oja 1994). The kinetics of the relaxation of the 820-nm absorbance change due to P-700$^+$ reduction is pseudo-first order. The recording of the absorbance change and its analysis to obtain the first-order rate constant for electron transport (k_e in s^{-1}) from the plastoquinol pool and P-700$^+$ were carried out as described in Kingston-Smith et al. (1999).

The CO_2 fixation and efficiency measurements were made in air consisting of 20% (v/v) oxygen (O_2), 350 ppm CO_2 with the remainder as nitrogen (N_2), at a temperature of 20–23°C. During experiments dark-adapted leaves were initially exposed to the lowest excitation irradiance to be used (50 μmol m^{-2} s^{-1}), and then to increasing levels of irradiance. An actinic light source was used to provide the step increase in irradiance from 0 to 750 μmol m^{-2} s^{-1} (light level stepn $\approx 2 \times$ light level step $_{(n-1)}$). Leaves were allowed to establish steady state photosynthesis, which took between 20 and 40 min, before photosynthetic measurements were made. A dark respiration measurement was made at the end of each irradiance step and all CO_2 fixation rates were calculated as gross rates. To investigate the possible development of sink-limitation of photosynthesis following herbicidal application, the photosynthetic measurements made at 20% (v/v) oxygen (irradiance from 0 to 750 μmol m^{-2} s^{-1}) were followed by measurements in 2% (v/v) oxygen (Foyer and Galtier 1996). Forty-five minutes after changing the oxygen concentration from 20% to 2%, during which time the leaf was in darkness, the photosynthetic parameters were measured again from lower to higher irradiance levels. Unsprayed 4-week-old (3-leaf stage) *S. nigrum* were measured at 1 and 3 DAT (no significant differences between 1 and 3 DAT, while treated *S. nigrum* plants were measured at 2 and 4 DAT. The quantum efficiency of gross CO_2 fixation (Φ_{CO_2}) at each irradiance level was calculated as the ratio of CO_2 fixation to incident irradiance.

The light-saturated rate of CO_2 fixation and the curvature factor of the irradiance response curves were calculated by fitting a non-rectangular hyperbola (Thornley and Johnson 1990):

$$A = \frac{\Phi \times I + A_{max} - [(\Phi \times I + A_{max})^2 - 4 \times \Phi \times I \times A_{max} \times \theta]^{1/2}}{2 \times \theta}$$

where A (μmol m^{-2} s^{-1}) is the CO_2 fixation rate, I (μmol m^{-2} s^{-1}) is the irradiance, Φ is an estimate of the maximal apparent quantum yield (note that this is different to Φ_{CO_2} measured under steady-state irradiance (see above)), A_{max} (μmol m^{-2} s^{-1}) is the light-saturated rate of photosynthesis at infinitely high irradiances and θ is the curvature term that describes the transition between the light-limited and light-saturated regions of the CO_2 fixation–irradiance curve. The non-rectangular hyperbolic model assumes that there is a completely non-linear relationship between CO_2 fixation and irradiance, an assumption that sometimes leads to an erroneous estimation of Φ. For that reason, the light-limited quantum yield of CO_2 fixation was more directly estimated as the difference between CO_2 fixation at zero irradiance and 50 μmol m^{-2} s^{-1} divided by the difference in irradiance (50 μmol m^{-2} s^{-1}).

Plant growth, leaf absorptance and chemical analysis

The growth of another set of identically grown plants (10 plants per treatment) was followed from 2 to 11 DAT. Leaves were separated into three classes: fully expanded (larger than 3 cm), partially expanded

(1–3 cm) and very small (smaller than 1 cm). Leaf development was assessed every 2–4 days. Plant height (from soil surface to plant tip), plant fresh weight and plant dry weight (after at least 48 h at 70°C) were determined at 11 DAT.

Another set of plants (four plants per treatment) was used for the determination of the leaf absorptance and leaf chlorophyll content. The absorptance of the third leaf (counted from bottom to top) in the spectral range 400–800 nm was measured at 2 nm intervals using a Taylor Sphere (for a non-diffuse incident irradiance) (LI-COR, Lincoln, Nebraska, USA) and an Instaspec CCD spectrometer (Oriel Scientific, Stratford, CT, USA). A weighed sample (0.1–0.2 g) from the third leaf was taken and the chlorophyll extracted with dimethylformamide. The extraction took 3–6 days in darkness at 4°C. Subsequently, the absorbance of the extract was measured with a spectrophotometer (Shimadzu UV 160-A; Shimadzu Scientific Instrument Corp., Columbia, Md., USA) at 647.0 and 664.5 nm. Chlorophyll concentration (Chl $a + b$ in mg g^{-1} FW) and chlorophyll *a/b* ratio were calculated (Inskeep and Bloom 1985).

Statistics

All measurements were conducted three times. Data were analysed at a significance level of $\alpha = 0.05$ with one-way ANOVA using Genstat 7.2 (Lawes Agricultural Trust, IACR-Rothamsted, UK).

Results

Plant growth

Plant growth was assessed from 0 to 11 DAT by counting the number of fully expanded leaves at 2–4 day intervals. Control and treated plants had a comparable number of fully expanded leaves until 2 DAT (Fig. 1). From 4 DAT onwards, control plants had significantly more fully expanded leaves than treated plants. The growth of treated plants was almost completely inhibited (3.1, 3.3, 3.3 leaves at 4, 8 and 11 DAT, respectively). At 11 DAT (final assessment) control plants had 6.5 leaves larger than 3 cm whereas treated plants had only 3.3. The dry weight and plant height of treated plant were also significantly reduced (Table 1). From these data it is evident that the ALS-inhibiting herbicide treatment had strong and rapid effects on the growth of *S. nigrum* plants.

Photosynthesis light response curves

At 20% O_2 concentration, the fixation of CO_2 by control plants increased with increasing irradiance up to the maximum irradiance employed (750 μmol m^{-2} s^{-1}). For treated plants (at both 2 and 4 DAT) CO_2 fixation approached light saturation at much lower irradiance (400 μmol m^{-2} s^{-1}, Fig. 2). At 20% O_2 concentration the maximum rate of CO_2 fixation, estimated by the non-rectangular hyperbola regression, was significantly higher for control plants (11.9 μmol m^{-2} s^{-1}) than for treated plants (5.0 and 2.8 μmol m^{-2} s^{-1} at 2 and 4 DAT, respectively). At both 2 and 4 DAT the difference in CO_2 fixation between control and treated plants was significant from 200 μmol m^{-2} s^{-1} onwards ($P < 0.05$). Reduction in atmospheric O_2 concentration from 20% to 2% produces an almost complete suppression of photorespiratory activity in leaves, and, consequently an increase in the rate of CO_2 fixation occurred (Fig. 2). The light-limited quantum yield of CO_2 fixation, estimated as a difference between CO_2 fixation at zero irradiance and 50 μmol m^{-2} s^{-1} divided by the difference in irradiance (50 μmol m^{-2} s^{-1}), was equal to 0.043 and 0.034 for O_2 concentrations of 20% to 2% with no significant differences between control and treated plants. The curvature factor (θ) was equal to 0.87 and 0.82 for O_2 concentrations of 20% to 2%, respectively with no significant differences between control and treated plants.

Quantum efficiency for electron transport by PSII and PSI

The quantum efficiency for electron transport by PSII at 660 nm wavelength ($\Phi_{PSII660nm}$) decreased with increasing irradiance at both atmospheric (20%) and

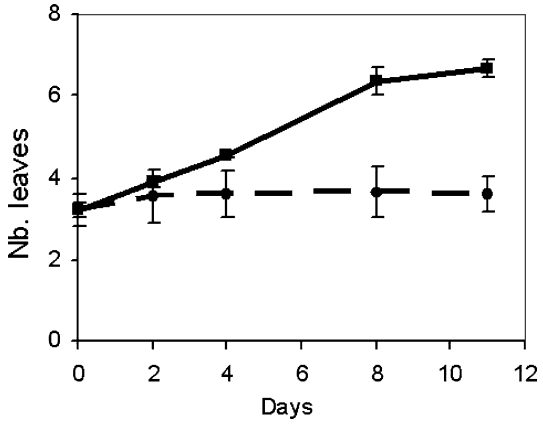

Fig. 1 Number of fully expanded leaves (per plant) plotted against days after treatment (DAT). Black line for control plants and grey dotted line for treated plants. Vertical bars indicate standard error of the mean

Table 1 Number of fully expanded leaves (per plant), dry weight (g per plant) and plant height (cm) for control plants and treated plant at final harvest (11 DAT)

	Number of fully expanded leaves	Dry weight	Plant height
Control	6.5 [a]	0.714 [a]	6.2 [a]
Treated	3.3 [b]	0.206 [b]	3.5 [b]
$LSD_{5\%}$	0.33	0.0594	0.28

For each measured parameter, numbers followed by different letters are significantly different ($P < 0.05$)

reduced (2%) oxygen concentrations (Fig. 3). As the relationship between $\Phi_{PSII660nm}$ and irradiance was not linear, the $\Phi_{PSII660nm}$ measurements of the different treatments were compared separately for each irradiance level. For both oxygen concentrations, $\Phi_{PSII660nm}$ decreased more with increasing irradiance in treated plants (at both 2 and 4 DAT) than in the control plants. The measurements at zero irradiance, equivalent to the dark-adapted ratio of variable/maximum fluorescence (F_v/F_m), were similar for both control and treated plants (at both 2 and 4 DAT) indicating the absence of photoinhibition at atmospheric oxygen concentration (Kingston-Smith et al. 1999). At atmospheric oxygen concentration, the difference in $\Phi_{PSII660nm}$ between the control and treated plants (at both 2 and 4 DAT) was significant at 200, 400 and 750 μmol m^{-2} s^{-1} ($P < 0.05$). At 2% oxygen concentration differences between control and treated plants (at both 2 and 4 DAT) were observed at 0, 100, 200 and 400 μmol m^{-2} s^{-1} ($P < 0.05$). No significant differences were recorded between the measurements of $\Phi_{PSII660nm}$ done at 2 DAT and at 4 DAT except in one occasion (2% O_2, 200 μmol m^{-2} s^{-1}). At high irradiance (750 μmol m^{-2} s^{-1}) the $\Phi_{PSII660nm}$ of both control and treated plants were low indicating progressive limitation of PSII efficiency due to a combination of increasing irradiance and a finite capacity for thylakoid electron transport. At high irradiance the photochemical efficiencies were low in both the control and treated leaves that it becomes difficult, at least at 2% O_2, to see any difference that could be attributed to the herbicide treatment. In the range of irradiances used the difference between $\Phi_{PSII660nm}$ values measured in 20% and 2% oxygen was small (Fig. 4) suggesting only a small down-regulation of electron transport in response to the inhibition of photorespiration.

Measurements of Φ_{PSI} provide similar information to $\Phi_{PSII,}$ but with additional information about the redox state of the PSI acceptor pool and the kinetics of the electron transfer between the photosystems (Harbinson and Hedley 1993). Similar to what was observed for $\Phi_{PSII660nm}$, Φ_{PSI} decreased with increasing irradiance (Fig. 5). This finding was previously reported (Genty et al. 1990; Harbinson et al. 1990b). In treated plants the decrease in Φ_{PSI} at both 2 and 4 DAT was stronger than in the control plants under both atmospheric and reduced oxygen concentrations. At atmospheric oxygen concentration, the difference in Φ_{PSI} between control and treated plants increased with the number of days after application of the herbicide. At 4 DAT, the difference in Φ_{PSI} between control and treated plants was significant at 100, 200 and 400 μmol m^{-2} s^{-1} ($P < 0.05$) but not at 750 μmol m^{-2} s^{-1}; whereas at 2 DAT, the difference in Φ_{PSI} between control and treated plants was only significant at 400 μmol m^{-2} s^{-1} ($P < 0.05$). Under reduced oxygen concentration, the difference in Φ_{PSI} between control and treated plants (at either 2 or 4 DAT) was significant from 50 to 400 μmol m^{-2} s^{-1} ($P < 0.05$) but not at 750 μmol m^{-2} s^{-1}. No significant differences were recorded between treated plants at 2 and 4 DAT. Measurements of Φ_{PSI} done at 20% oxygen were 10–15% higher than measurements obtained at 2% oxygen concentrations.

The predominantly linear relationship between $\Phi_{PSII560nm}$ and Φ_{PSI} (Fig. 6), passing close by the origin,

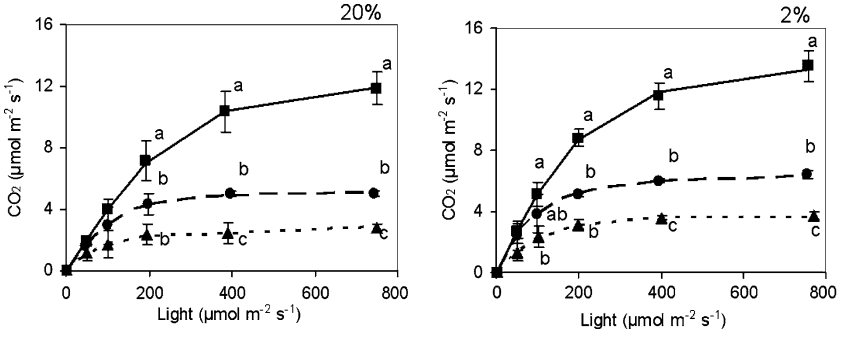

Fig. 2 Relationship between CO_2 fixation (μmol m^{-2} s^{-1}) and irradiance (μmol m^{-2} s^{-1}) at 20% and 2% oxygen for control plants (black squares) and treated plants at 2 DAT (grey circles) and 4 DAT (grey triangles). Vertical bars indicate standard error of the mean. Non-rectangular hyperbola fit for control (black line), treated plants at 2 DAT (solid grey line) and 4 DAT (dotted grey line)

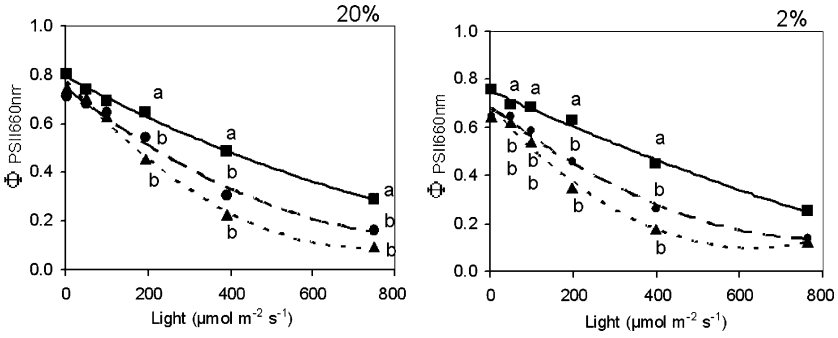

Fig. 3 Relationship between $\Phi_{PSII660\ nm}$ and irradiance (μmol m^{-2} s^{-1}) at 20% oxygen and 2% oxygen for control (black squares), treated plants at 2 DAT (grey circles) and 4 DAT (grey triangles). For each irradiance level (*) indicates that measurements for control plants are significantly different than measurements for treated plants ($P < 0.05$)

demonstrates that over a wide range of irradiance (from 100 to 750 μmol m^{-2} s^{-1}), oxygen concentrations (20% or 2%) and plant status (control or treated with an ALS-inhibiting herbicide), the plants maintain a close co-ordination between the quantum efficiencies of PSI and PSII in relation to changing capacities for CO_2 fixation. Figure 7 shows the relationship between the values of Φ_{CO_2} obtained from treated (2 and 4 DAT) and untreated *S. nigrum* leaves under non-photorespiratory and photorespiratory conditions. Clearly the quantum yield of CO_2 fixation under photorespiratory condition is linearly related to the yield under non-photorespiratory conditions over the greater part of a range of values obtained, and there appears to be no significant difference between treated and untreated leaves in respect of the response of their CO_2 fixation efficiency to the elimination of photorespiration.

The relationship between CO_2 fixation and the index of linear photosynthetic electron transport through PSII, J_{PSII} (which is the product of Φ and irradiance) is presented in Fig. 8. The J_{PSII} and J_{PSI} (index of linear photosynthetic electron transport through PSI which is the product of Φ_{PSI} and irradiance, data not shown) give a 'rate' of electron transport which is proportional to the rate of photosynthetic electron transport through PSII or PSI (Kingston-Smith et al. 1997, 1999). In the range of irradiance tested the relationships between $J_{PSII660nm}$, J_{PSI} (data not shown) and CO_2 fixation were linear under both atmospheric and reduced oxygen concentrations. Surprisingly, the slopes of the relationship between $J_{PSII660nm}$, J_{PSI} (data not shown) and CO_2 fixation were different for control and treated plants at 2 and 4 DAT irrespective of the oxygen concentration.

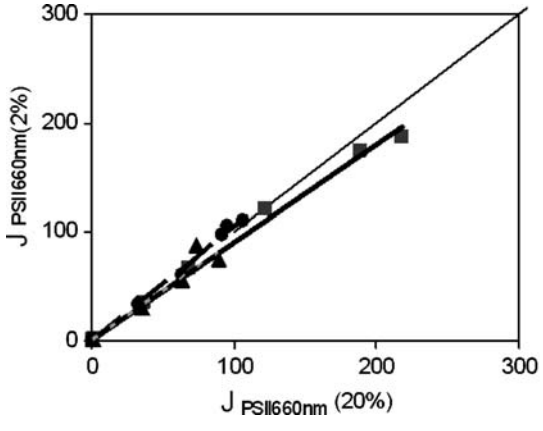

Fig. 4 Relationship between $\Phi_{PSII660\ nm}$ at 2% oxygen and $\Phi_{PSII660nm}$ at 20% oxygen for control (black squares), treated plants at 2 DAT (grey circles) and treated plants at 4 DAT (grey triangles). The trend lines are linear regressions constrained to pass through the origin. A 1:1 trend line has also been drawn

The apparent rate constant for photosynthetic electron transport was independent of irradiance in the range 200–750 μmol m^{-2} s^{-1}. No significant differences were recorded between control and treated plants exposed to 20% oxygen concentrations. Comparable values were obtained for plants exposed to 2% oxygen. The ΔA_{820nm} parameter corresponding to complete oxidation of P700 ($\Delta A_{820nmmax}$) was higher for control plants (around 84 arbitrary units) than for treated plants (43.5 and 41 arbitrary units at 2 and 4 DAS, respectively) and rather stable over the range of irradiance and oxygen concentrations tested. The maximum rate of CO_2 fixation appeared to be substantially independent of the apparent rate constant for P700 reduction following a light / dark transition at both 20 and 2% oxygen concentrations (Fig. 9). At 20% O_2, rate constant measurements were 10–27% larger than measurements done at 2% O_2.

Chlorophyll content

Compared to control plants the level of chlorophyll $a + b$ was significantly lower in treated plants

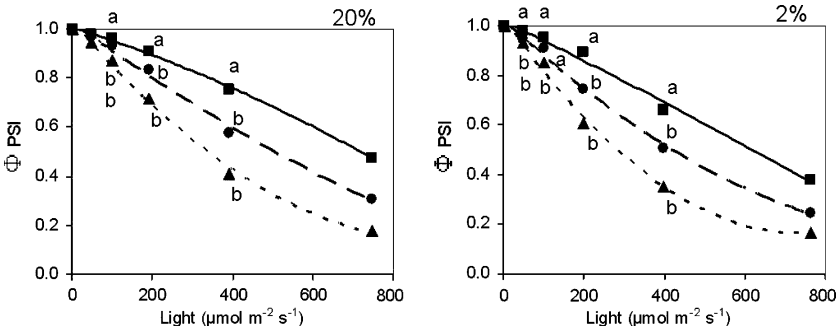

Fig. 5 Relationship between Φ_{PSI} and irradiance (μmol m^{-2} s^{-1}) at 20% oxygen and 2% oxygen for control (black squares), treated plants at 2 DAT (grey circles) and 4 DAT (grey triangles). For each irradiance level (*) indicates that measurements for control plants are significantly different than measurements for treated plants ($P < 0.05$)

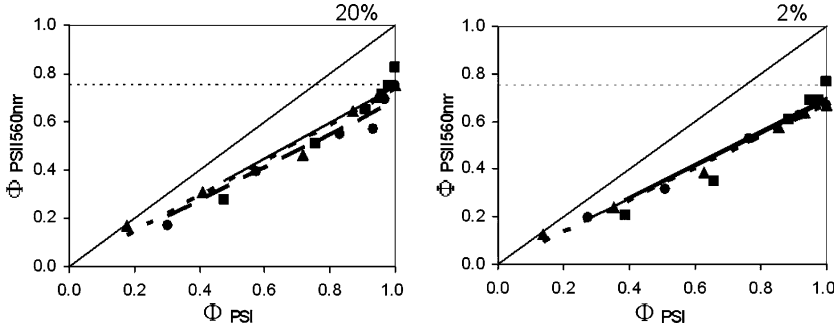

Fig. 6 Relationship between $\Phi_{PSII560\ nm}$ and Φ_{PSI} at 20% oxygen 2% oxygen for control (black squares), treated plants at 2 DAT (grey circles) and 4 DAT (grey triangles). The trend lines are linear regressions constrained to pass through the origin. A 1:1 trend line has also been drawn

(Table 2). Already at 2 DAT the total chlorophyll concentration of treated plants (Treated, 2 DAT) was reduced by 25%. At 4 DAT the chlorophyll content of treated plants (Treated, 4 DAT) was 45% lower than of control plants (Control, 4 DAT). No significant differences were observed between control plants at 2 and 4 DAT. After herbicide treatment a slight change in chlorophyll *a*/*b* ratio was observed (Table 2). The chlorophyll *a/b* ratio of treated plants (3.376 and 3.364 at 2 and 4 DAT, respectively) was lower than that of control plants (3.600 and 3.526 at 2 and 4 DAT, respectively). This change indicates that in the treated plants the relative loss of chlorophyll *a* was only slightly greater than the loss of chlorophyll *b*. Positive linear relationships were observed between both the maximum rate of CO_2 fixation (Fig. 10) or the $\Delta A_{820nmmax}$ parameter (Fig. 11) and the concentration in chlorophyll $a + b$ present into the plants.

Leaf absorptance versus chlorophyll content

The herbicide treatment had little effects on leaf absorptance. The reduction in leaf absorptance was in average 5% for treated plants at 2 DAT and 10% for treated plants at 4 DAT. A linear relationship was found between absorptance and the total chlorophyll concentration (data not shown).

Discussion

Herbicide effects on plant growth

The application of the herbicide metsulfuron-methyl produced a strong and rapid reduction in the growth of *S. nigrum* plants. Data presented suggest a very short time lag before an effect on growth was apparent. This

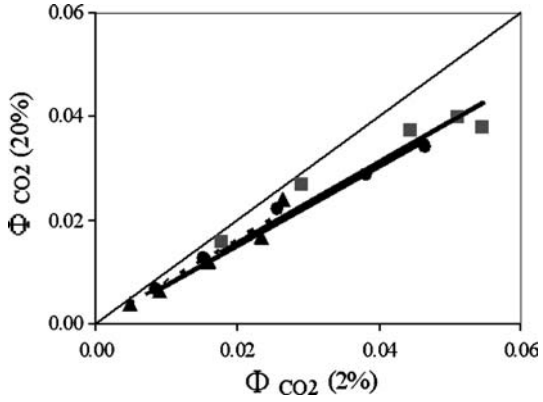

Fig. 7 Relationship between the quantum yield of CO_2 fixation (Φ_{CO_2}) at 20% oxygen and quantum yield of CO_2 fixation (Φ_{CO_2}) at 2% oxygen for control (black squares), treated plants at 2 DAT (grey circles) and treated plants at 4 DAT (grey triangles). The linear regression trend lines were forced to go through the origin and a 1:1 line has been drawn to

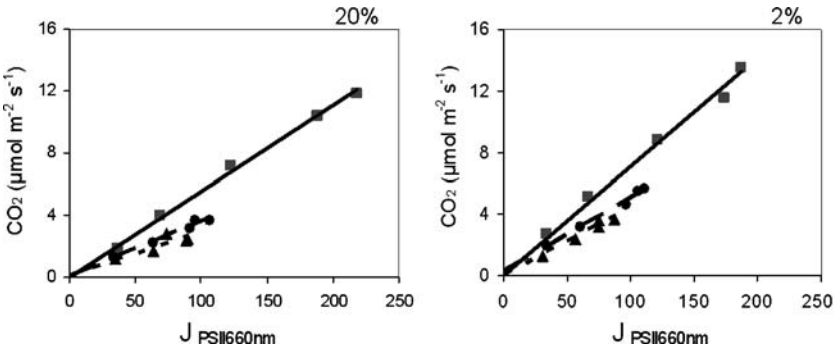

Fig. 8 Relationship between CO_2 fixation (µmol m^{-2} s^{-1}) and $J_{PSII660nm}$ at 20% oxygen and 2% oxygen for control plants (black squares), treated plants at 2 DAT (grey circles) and 4 DAT (grey triangles). The linear regression trend lines were forced to go through the origin

is consistent with results from Gaston et al. (2003) and earlier observations from Shaner and Singh (1992) that the growth of plants treated with ALS-inhibiting herbicides such as sulfonylurea, slows within hours and ceases within a few days. At final harvest, treated plants exhibited stunting that is known to be one possible ALS-inhibiting-herbicide symptom (Peterson 2001). As expected, treated plants also exhibited interveinal chlorosis, red leaf venation, purpling and gradual death. The question arises as to how this cessation of growth relates to changes in the operation of photosynthesis, and especially whether growth limits photosynthesis or vice versa.

The relationship between the herbicide, photosynthesis and growth

It is evident that even though methsulfuron-methyl is not a herbicide that targets the photosynthetic machinery, its application produces a rapid progressive reduction in photosynthesis, with both CO_2 fixation (Fig. 2) and electron transport showing a reduction in activity following application of the herbicide. Given the rapid cessation of growth this might not appear surprising. However, the absence of the any effect of herbicide application on the relative efficiencies of CO_2 fixation at 20% and 2% O_2 suggests that the down-regulation of CO_2 fixation was not due to sink-limitation. If sink limitation of photosynthesis would have developed, it would be expected to produce some degree of oxygen insensitivity in the operation of CO_2 fixation. This would have revealed itself as a reduced increase in the rate of CO_2 fixation following the elimination of photorespiration by using an atmosphere containing 2% O_2 (Foyer and Galtier 1996). The absence of sink limitation is supported by the lack of any effect of herbicide application on the Φ_{PSII} or J_{PSII} irradiance response measured on leaves in either 2% or 20% O_2 (Fig. 3). Regardless of the whether or not the plants had been treated with herbicide, the values of Φ_{PSII} or J_{PSII} obtained over a range of irradiances were only slightly lower in 2% O_2 compared to 20% (Fig. 4). This means that photosynthetic electron transport was only slightly down-regulated by the elimination of photorespiration, a response that implies only minor sink-limitation in either the control or treated plants. Though it appears that the reduction in photosynthesis was not due to the restriction of growth, it cannot, however, be inferred from these data that the reverse was true. The reduction of growth is rapid but the reduction in photosynthetic capacity was more gradual, so it may be that the inhibition of photosynthesis

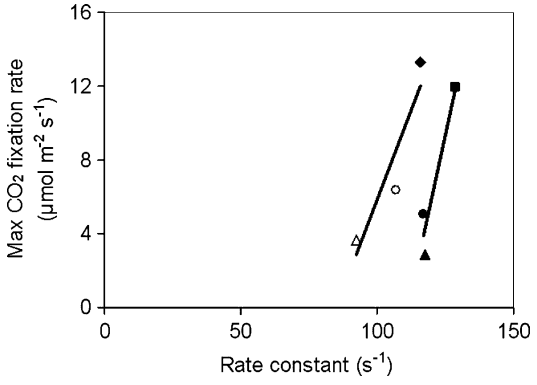

Fig. 9 Maximum CO_2 fixation (µmol m^{-2} s^{-1}) and the apparent rate constant for P700 reduction (k_e in s^{-1}) following a light/dark transition at 20% (full symbols) and 2% oxygen (empty symbols) for control plants (black squares), treated plants at 2 DAT (grey circles) and 4 DAT (grey triangles). The trend lines are linear regressions

Table 2 Chlorophyll concentration (Chl $a + b$ in mg g^{-1} fresh weight) and chlorophyll a/b ratio for control plants and treated plants at 2 and 4 days after treatment

Treatments	DAT	Chl $a + b$	Chl a/b
Control	2	1.705 [a]	3.600 [a]
Control	4	1.760 [a]	3.526 [a]
Treated	2	1.272 [b]	3.376 [b]
Treated	4	0.992 [c]	3.364 [b]
$LSD_{5\%}$		0.0896	0.0877

Numbers followed by different letters are significantly different ($P < 0.05$)

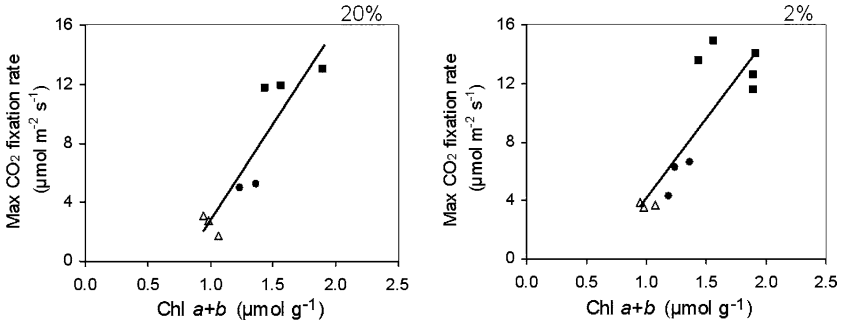

Fig. 10 The relationship between maximum CO_2 fixation (μmol m^{-2} s^{-1}) and chlorophyll $a + b$ concentration for leaves exposed to 20% ($r^2 = 0.82$) and 2% ($r^2 = 0.74$) oxygen. Control plants (black squares), treated plants at 2 DAT (grey circles) and treated plants at 4 DAT (grey triangles). The trend-lines are linear regressions

and growth were not directly dependent on each other, though sharing a common cause.

Herbicidal treatment and the internal co-ordination of PSI and PSII activity

The irradiance dependencies of both Φ_{PSII} and Φ_{PSI} are strongly affected by herbicidal treatment (Figs. 3, 5). However, the relationship between the efficiencies of both photosystems, whether revealed by comparing the changing efficiencies or electron fluxes, is unaltered by herbicidal treatment (Fig. 6). The efficiencies of both photosystems decline in parallel, suggesting a predominant role for linear electron transport—a phenomenon that has been widely reported (Harbinson and Foyer 1991; Harbinson et al. 1990a; Kingston-Smith et al. 1999). It is noteworthy that the consistent, linear relationship between the efficiencies of both photosystems is sustained under conditions where large changes are occurring in the pigment composition of the leaves (Table 2). This loss of pigments is not accompanied by any photoinhibition of PSII, and the value of $\Delta A_{820nmmax}$ value decreases in proportion to the decreasing leaf chlorophyll content (Fig. 11). The chlorophyll *a/b* ratio is also largely unchanged by the effects of the herbicide (Table 2). Taken together these data imply that the loss of chlorophyll is sufficiently organised so as to maintain the balance of pigment-protein complexes and function between the photosystems close to that found in control leaves. This situation is similar to that found in cold-grown maize leaves, where in spite of large reductions in leaf chlorophyll provoked by prolonged exposure to low temperatures, the balance between photosystem I and photosystem II function was maintained (Kingston-Smith et al. 1999). It appears that the stress-induced loss of leaf chlorophyll is not a catastrophic, uncontrolled process, but rather the consequence of a well organised breakdown of components.

The rate constant data are especially interesting with regards to the loss of photosynthetic activity of the leaves. Though light saturated CO_2 fixation rates are progressively decreased by herbicidal activity, the values of the rate constant for $P700^+$ reduction by electrons coming from PQH_2 pool (k_e) remain relatively unchanged. The apparent contradiction between these observations of the relationship observed here and those reported previously can be partly explained by

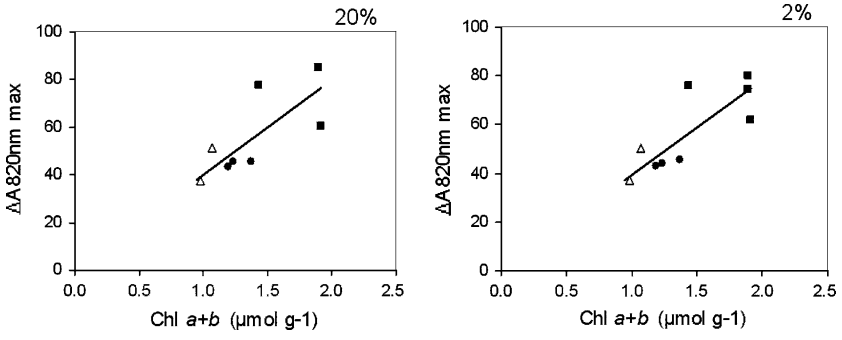

Fig. 11 The relationship between $\Delta A820nm$ corresponding to complete oxidation of P700 and chlorophyll $a + b$ concentration for leaves exposed to 20% ($r^2 = 0.54$) and 2% ($r^2 = 0.65$) oxygen. Control plants (black squares), treated plants at 2 DAT (grey circles) and treated plants at 4 DAT (grey triangles)

the changing leaf chlorophyll contents. The actual rate of electron transport associated with a particular rate constant will depend on the quantity of P700 present in the leaf. If the P700 pool decreases then an unchanging rate constant will create a decreasing rate of electron transport. The decrease in chlorophyll content is associated with a decrease in the $\Delta A_{820nmmax}$; the latter reflects the amount of P700 present in the leaf. The decrease in leaf chlorophyll observed during experiments, though large, was insufficient to produce any significant change in leaf absorbance. This implies that the changes in electron transport that occur while k_e remains constant, cannot be attributed to a loss of overall light-trapping by the leaves. It may even be that the stability of the rate-constant is the goal of the control process that modifies leaf chlorophyll during the stress created by the herbicide application.

The relationship between electron transport and CO_2 fixation

The linear relationship between gross CO_2 fixation and the index of linear photosynthetic electron transport through PSI (J_{PSI}) or PSII (J_{PSII}) observed for plants exposed to non-photorespiratory conditions is in agreements with the findings of Harbinson et al. (1990a). Herbicidal treatment, however, affects the quantitative relationship between electron transport and gross CO_2 fixation. In both photorespiratory and non-photorespiratory conditions, the relationship between the index of electron flow through photosystem I or II, and the rate of CO_2 fixation is altered so that electron transport becomes less efficient at driving CO_2 fixation (Fig. 8). The effect occurs even under non-photorespiratory conditions so it is not due to an increase in photorespiration provoked by the herbicidal treatment. It also cannot be due to a change in leaf absorbance, as this is scarcely changed by the herbicidal treatment. It cannot likewise be due to the loss of chlorophyll acting in some other unexpected way, as the effects were the same at 2 and at 4 DAT, whereas a large drop in chlorophyll content was observed between 2 and 4 DAT. One simple explanation is that the loss of efficiency of electron transport with respect to CO_2 fixation is due to the development of another sink for electron transport. It cannot be stated what this sink is, though speculation that it is O_2 is inevitable. If O_2 were the acceptor this could be a factor in the herbicidal effect produced by metsulfuron-methyl. Considering the response in more detail, a change in atmospheric O_2 concentration from 2% to 20% produces a 22% drop in the rate of CO_2 fixation of control leaves measured when J_{PSII} is 100. In treated leaves under the same circumstances the drop was 28% (Fig. 8). So though there appears to be some alternate acceptor activity in the treated leaves, the electron transport–CO_2 relationship is still responding to changes in O_2 concentration in a way that is broadly consistent with the existence of photorespiration.

Early detection of the activity of ALS-inhibiting herbicides on weeds

As results clearly demonstrated difference in carbon dioxide fixation, the quantum efficiency for electron transport by PSII (Φ_{PSII}) or PSI (Φ_{PSI}) and the total chlorophyll content between control and treated *S. nigrum* plants, early detection of the activity of ALS-inhibiting herbicides on weeds seemed practical. Further experiments confirmed the present results for both *S. nigrum* and *Polygonum persicaria* plants grown under greenhouse and *S. nigrum* plants grown under field conditions. Possibilities of using the different photosynthesis related parameter to assess the effect of an ALS-inhibiting herbicide are discussed elsewhere.

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