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Can photosynthesis-related parameters be used to establish the activity of acetolactate synthase–inhibiting herbicides on weeds?

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The application of the acetolactate synthase (ALS)–inhibiting herbicide metsulfuron on greenhouse- and field-grown black nightshade and greenhouse-grown ladysthumb resulted in progressive inhibition of the level of carbon dioxide (CO_2) fixation, the relative quantum efficiency of electron transport through photosystem I (Φ_{PSI}) and II (Φ_{PSII}), and the leaf chlorophyll content. Photosynthetic-related measurements, measured 2 to 4 d after treatment (DAT) at photon flux densities of 400 to 500 μmol m^{-2} s^{-1}, provided valuable information before the visual symptoms that first appeared at 7 to 10 DAT with the herbicide. Measurements of the quantum efficiency for electron transport by photosystem II and the loss in leaf chlorophyll content appeared to be two of the most practical parameters to use when designing an early detection method to assess the toxicity of metsulfuron. The use of chlorophyll fluorescence would require a comparison of steady-state Φ_{PSII} measurements for control and treated plants, which could be realized by either measuring in time (before/after application) or space (treated/untreated patch).

Nomenclature: Metsulfuron; black nightshade, *Solanum nigrum* L. SOLNI; ladysthumb, *Polygonum persicaria* L. POLPE.

Key words: ALS, chlorophyll, fluorescence, photosynthesis.

In 2000 to 2001, herbicides accounted for almost 40% of the pesticide use worldwide in terms of the volume of active ingredient (EPA 2004). Increased concerns about the environmental side effects of herbicides, the development of herbicide resistance in weeds, and the economic drive to reduce the cost of the inputs have resulted in increasing pressure on farmers to reduce the use of herbicides. The minimum lethal herbicide dose (MLHD) technology (Kempenaar et al. 2002; Ketel 1996) has shown itself to be a promising decision support system, leading to the use of lower rates of photosynthesis-inhibiting herbicides. This method calculates the minimum dose of a photosynthesis-inhibiting herbicide needed to control a weed population. A method for the early detection of herbicidal effect, based on simple and rapid measurements of either photosystem I or photosystem II activities, is then used to evaluate the efficacy of the treatment shortly after application. Extension of the MLHD technology to other common groups of herbicides requires the identification of suitable parameters for evaluating the activity of the herbicides shortly after application.

Developed over the past 20 yr, the acetolactate synthase (ALS) inhibitors are an increasingly important group of herbicides. ALS-inhibiting herbicides are widely used around the world because of the relatively low application rates required and their limited environmental impact, low mammalian toxicity, wide crop selectivity, and high efficacy (Peterson 2001). These herbicides selectively inhibit ALS (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. Metabolic and physiological responses to sulfonylureas include a rapid cessation of growth, which occurs within hours after the application (Gaston et al. 2003; Rhodes et al. 1987; Shaner and Singh 1991), the inhibition of the mitosis and DNA synthesis, the increase in free amino acids, the rapid decrease in the level of soluble protein, and a decrease in the translocation of photosynthate to the growing points of the plant (Devine 1989, cited in Shaner and Singh 1992). 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 been observed in treated plants. Judy et al. (1990) found effects on fluorescence of barley (*Hordeum vulgare* L.) 2 h after treatment with imazaquin. Percival and Baker (1991) found effects on the fluorescence of wheat leaves 24 h after application of the ALS-inhibitor imazamethabenz at its recommended rate. Barbagallo et al.

(2003) also demonstrated that many inhibitors of metabolic processes that are not directly involved in photosynthetic metabolism can produce modifications to fluorescence kinetics. In an extensive study, Riethmuller-Haage et al. (2006) showed that the rate of carbon dioxide (CO_2) fixation, the relative quantum efficiency of photosystem II electron transport (Φ_{PSII}), the relative quantum efficiency of photosystem I electron transport (Φ_{PSI}), and the total chlorophyll content of black nightshade plants were all significantly reduced after application of the ALS-inhibiting herbicides metsulfuron. These last observations were made on climate chamber–grown plants.

The extension of the MLHD technology to the ALS inhibitors requires the identification of suitable parameters for evaluating the activity of the herbicides shortly after application. Once we clearly established that the rapid changes in the operation of photosynthesis after application of metsulfuron observed on climate chamber–grown black nightshade plants were also detectable for black nightshade plants grown under more natural conditions and for ladysthumb, a second weed species, the possibility of using a photosynthesis-related parameter to assess the effect of an ALS-inhibiting herbicides on weeds was determined. Further investigations will ensure that the selected parameters are appropriate for determining differences in efficacy between lethal and sublethal rates of herbicides. This aspect is beyond the scope of the present article.

Materials and Methods

Plant Material and Spraying Procedure

Seeds of black nightshade or ladysthumb were put on a tray containing a moistened mixture of potting soil and sand (2 : 1), which was placed in a climate chamber at 22/18 C day/night temperatures and 70% relative humidity for 15 d. After germination, individual weed seedlings were transferred into 1 dm^3 pots and, depending on the trial, placed in a climate chamber, in a greenhouse, or outside. For the first trial (S-1), which took place in October 2003, black nightshade plants were grown in a climate chamber at 22/18 C day/night temperature and 70% relative humidity. The photon flux density was 220 μmol m^{-2} s^{-1} for 12 h produced by TL-D-HF lamps.[1] Two trials were conducted in February 2004. Black nightshade (trial S-2) and ladysthumb plants (trial P-2) were grown in a greenhouse at 18/14 C day/night temperatures, 70% relative humidity, and a 12-h photoperiod provided by natural light supplemented with high-pressure mercury lamps. For the last trial (trial S-3), black nightshade plants were grown in the field between April 29, 2004, and June 18, 2004. The average temperature was 15.5 C (maximum 30.6, minimum 4.9 C). Weed plants rested on an irrigation mat and were irrigated daily.

Plants of black nightshade and ladysthumb were always sprayed in their three-leaf stage, with an air-pressurized laboratory track sprayer, delivering 400 L ha^{-1} of herbicide solution at 303 kPa. Black nightshade plants were treated with 16 g ai ha^{-1} of metsulfuron and 0.75% v/v isodecyl ethoxylate. A preliminary test showed that the recommended field rate for metsulfuron (8 g ai ha^{-1}) was insufficient to kill growth chamber–raised black nightshade plants. For that reason, an application rate of 16 g ai ha^{-1} was used as standard in the various trials (S-1, S-2, P-2, and S-3). For black nightshade plants grown under field conditions (trial S-3) both 16 and 8 g ai ha^{-1} were tested, and no significant differences were observed (data not shown).

Photosynthesis Measurements

To have an indication of the relative performance of the photosynthetic apparatus of ALS-treated plants, the level of CO_2 fixation, the relative quantum efficiency of Φ_{PSII}, and the relative quantum efficiency of Φ_{PSI} were assessed for both control and treated plants (Riethmuller-Haage et al. 2006). Equipment essentially identical to that described by De Groot et al. (2003) was used. CO_2 fixation was measured using an infrared gas analyzer.[2] Actinic light was provided by a quartz halogen lamp filtered by near infrared (NIR) and Calfex dichroic mirrors,[3] and light-intensity was adjusted using metal film neutral-density filters[4] (De Groot et al. 2003). A wavelength of 660 nm was used to excite the chlorophyll fluorescence to measure the relative quantum efficiency of Φ_{PSII}. The relative quantum efficiency of Φ_{PSI} was measured using the irradiance-induced absorbance change around 820 nm (Harbinson and Woodward 1987).

The CO_2 fixation and efficiency measurements were made in air consisting of 20% (v/v) oxygen (O_2) and 350 ppm CO_2, with the remainder nitrogen (N_2) at a temperature of 20 to 23 C. During experiments dark-adapted leaves were initially exposed to the lowest excitation irradiance level (50 μmol m^{-2} s^{-1}) and then to increasing levels of irradiance. The actinic light source was controlled to provide a step-wise increase in photon flux density from 0 to 800 μmol m^{-2} s^{-1}. At each irradiance level, leaves were allowed to establish steady state photosynthesis, which took between 20 to 40 min, before the measurements of CO_2 fixation, Φ_{PSII}, and Φ_{PSI} were made. Unsprayed and treated black nightshade plants at the three-leaf stage were measured at 2 and 4 d after treatment (DAT) (trials S-1 and S-2). Black nightshade plants grown in the field (trial S-3) were measured at 2, 4, and 7 DAT, and ladysthumb plants (trial P-2) were measured at 2 DAT.

Plant Growth and Chemical Analysis

Plant height (from soil surface to plant tip) and plant dry weight (after at least 48 h at 70 C) were determined at 11 (trial S-1), 14 (trials S-2 and P-2), or 15 DAT (trial S-3). Additionally, in trials S-1 and S-3, the number of fully expanded leaves (larger than 3 cm) was assessed every 2 to 4 d, from application day to final harvest. Four plants per treatment were used for chlorophyll extraction at 2 and 4 DAT (trial S-1) or 2, 4, and 7 DAT (trial S-3). A sample (0.1 to 0.2 g fresh weight) from the third leaf was taken, and chlorophyll content was extracted in 3 ml dimethylformamide. The extraction, carried out in darkness, took 3 to 6 d at 4 C. Subsequently, the absorbance of the chlorophyll solution was measured with a spectrophotometer[5] at 647.0 and 664.5 nm. The chlorophyll concentration (Chl $a + b$ in mg g^{-1} fresh weight) and chlorophyll ratio (a/b) were calculated according to Inskeep et al. (1985).

Statistics

Each trial was conducted in three replications. Data were analyzed with one-way ANOVA using Genstat 7.2.[6] Differ-

TABLE 1. Dry weight and plant height of black nightshade (SOLNI) or ladysthumb (POLPE) plants treated with the acetolactate synthase (ALS)-inhibiting herbicide metsulfuron. Plants were grown in a climate chamber (CC), a greenhouse (GH), or under field conditions (Field). Measurements were taken at 11 d after treatment (DAT) (S-1), 14 DAT (S-2 and P-2), and 15 DAT (S-3). Treatment measures followed by different letters indicate significant (alpha = 0.005) treatment effects.

Trial	Species	Growth	Dry weight (g)			Plant height (cm)		
			Control	Treated	% diff.	Control	Treated	% diff.
			g			cm		
S-1	SOLNI	CC	0.714 a	0.206 b	71.1	6.2 a	3.5 b	43.5
S-2	SOLNI	GH	0.625 a	0.209 b	66.6	5.7 a	3.7 b	35.0
S-3	SOLNI	Field	0.382 a	0.040 b	89.5	4.75 a	1.35 b	71.6
P-2	POLPE	GH	1.160 a	0.696 b	40.2	12.1 a	8.5 b	30.0

ences between means were evaluated at a significance level of $\alpha = 0.05$.

Results

Plant Growth

In all trials, application of metsulfuron resulted in significant reductions in plant dry weight and plant height. The dry weight of treated black nightshade plants was significantly reduced from 67% (trial S-2) to 90% (trial S-3). Plant height was also significantly reduced because treated black nightshade plants were, on average, 35% (trial S-2) to 72% (trial S-3) smaller than control plants. Treated ladysthumb plants grown in the greenhouse (trial P-2) had their dry weight reduced by 40% and their plant height reduced by 30% (Table 1). Very soon after application of the herbicide, the number of fully expanded leaves on treated plants stayed less than that observed on control plants (Figure 1). The number of fully expanded leaves on treated plants was completely inhibited at 4 (trial S-1) and 7 DAT (trial S-3) and afterward.

As expected, both treated black nightshade and ladysthumb plants exhibited chlorosis, red leaf venation, purpling, and gradual death (Peterson 2001). For climate chamber–and greenhouse-grown black nightshade (trials S-1 and S-2) and ladysthumb plants (trial P-2), visual symptoms were first noticeable 4 to 7 DAT, and death of treated plants occurred at 2 to 3 wk after application of the herbicide. For black nightshade plants grown under field conditions (trial S-3), the time lag before visual symptoms appeared was slightly longer because symptoms were first noticeable 10 DAT. Death of treated plants occurred 3 to 4 wk after application of the herbicide.

Photosynthesis Light-Response Curves

The fixation of CO_2 by control plants increased with increasing irradiance (Figure 2). For control plants grown in the climate chamber (trial S-1) or in the greenhouse (trial S-2 and P-2), CO_2 fixation approached light saturation at around 750 μmol m^{-2} s^{-1}, with maximum rates of CO_2 fixation at around 13 and 5 μmol m^{-2} s^{-1}, respectively. Untreated black nightshade plants grown in the field (trial S-3), which had acclimated to higher intensities, were clearly not close to light saturation at the highest irradiance used (750 μmol m^{-2} s^{-1}). At that irradiance level, a CO_2 fixation rate of around 20 μmol m^{-2} s^{-1} was obtained.

The maximum rate of CO_2 fixation by treated black nightshade and ladysthumb plants decreased as the number of days after treatment increased. Treated plants were more affected at 7 DAT or 4 DAT than at 2 DAT (Figure 2). Treated plants also approached light saturation at a much lower irradiance level (400 μmol m^{-2} s^{-1}). For black nightshade plants grown in a climate chamber (trial S-1), the differences in CO_2 fixation between control and treated black nightshade plants were significant at a light intensity of 200 μmol m^{-2} s^{-1} and higher. The maximum rate of CO_2 fixation of treated plants at 750 μmol m^{-2} s^{-1} was

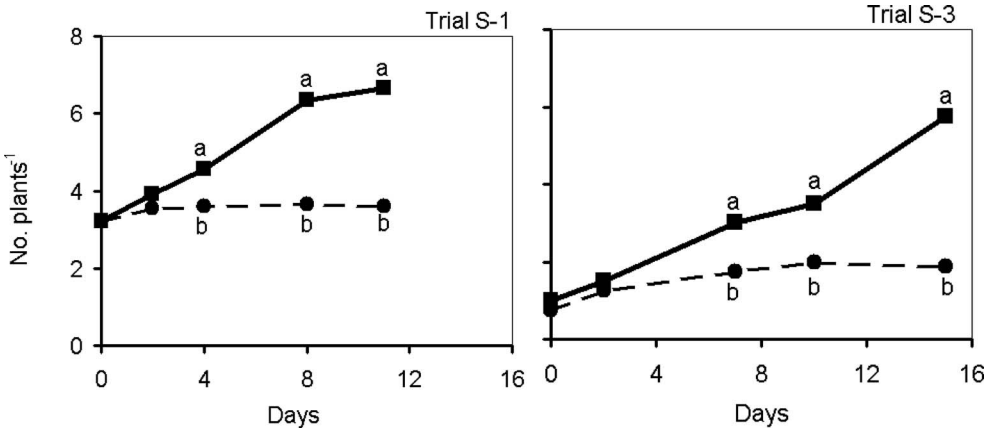

FIGURE 1. Number of fully expanded leaves (per plant) plotted against time after treatment (days) for black nightshade plants grown under climate chamber conditions (S-1) or under field conditions (S-3). Solid line represents control plants, and dashed line represents plants treated with the acetolactate synthase (ALS)-inhibiting herbicide metsulfuron. Treatment measures followed by different letters indicate significant (alpha = 0.05) treatment effects.

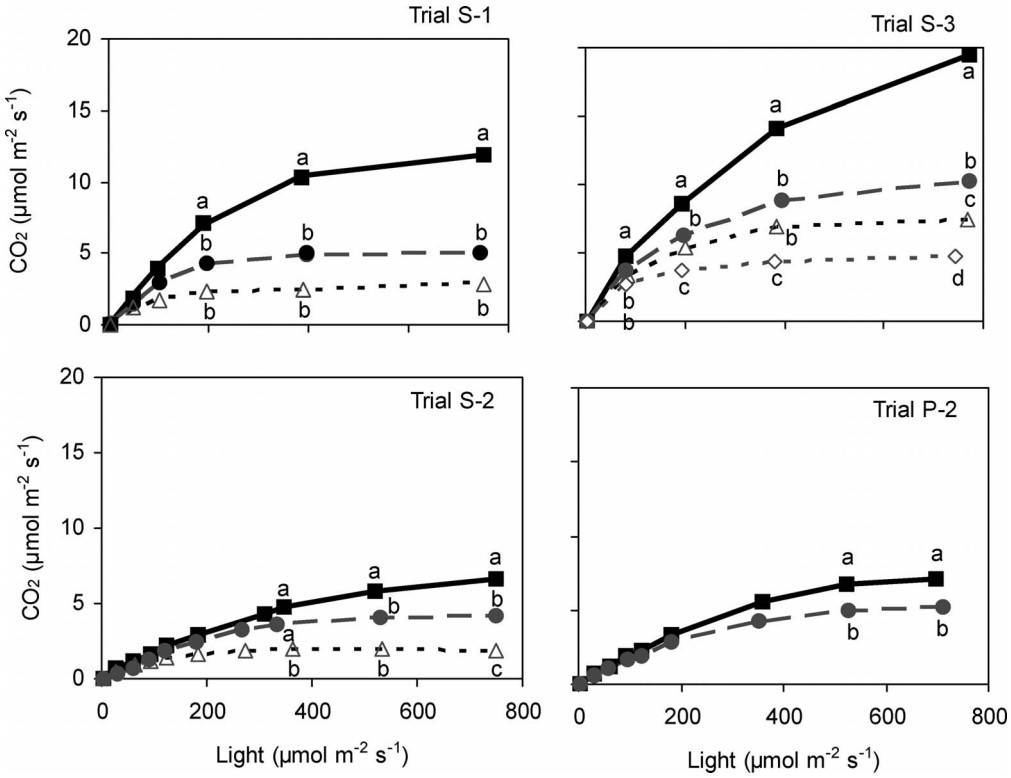

FIGURE 2. Relationship between CO_2 fixation (µmol m^{-2} s^{-1}) and irradiance (µmol m^{-2} s^{-1}) for black nightshade plants grown in a climate chamber (S-1), a greenhouse (S-2), and under field conditions (S-3) and ladysthumb plants grown in a greenhouse (P-2). Data for control plants (■, solid line) and metsulfuron-treated plants at 2 d after treatment (DAT) (●, dashed line), 4 DAT (△, dotted line), and 7 DAT (◇, dotted line). Treatment measures followed by different letters indicate significant (alpha = 0.05) treatment effects.

reduced by 58% at 2 DAT and 76% at 4 DAT (Figure 2, S-1). For black nightshade plants grown in the greenhouse (trial S-2), a nearly similar reduction of the maximum rate of CO_2 fixation (70%) was recorded at 4 DAT (Figure 2, S-2). The lowest light intensity at which control and treated plants could be significantly differentiated was around 350 µmol m^{-2} s^{-1}, which was close to the light intensity needed in the case of climate chamber–grown plants (trial S-1). When black nightshade plants were grown outside (trial S-3), the time required to produce a 70% reduction of the maximum rate of CO_2 fixation was slightly longer (7 d). However, by that time, significant differences between control and treated plants could already be observed from a light intensity of 80 µmol m^{-2} s^{-1} and higher (Figure 2, S-3). For ladysthumb plants grown in the greenhouse (trial P-2), the maximum rate of CO_2 fixation was reduced by 40% at 2 DAT, which was in line with observations for greenhouse-grown black nightshade plants. The lowest light intensity at which control and treated plants could be differentiated was around 500 µmol m^{-2} s^{-1}, which was slightly higher than that for black nightshade plants (Figure 2, P-2).

Quantum Efficiency for Electron Transport by PSII and PSI

The quantum efficiency for Φ_{PSII} was measured at 660 nm wavelength at a photon flux density of 0 µmol m^{-2} s^{-1}, equivalent to a dark-adapted chlorophyll fluoresence ratio (Fv/Fm), were around 0.8 for both control and treated black nightshade and ladysthumb plants, at all observation dates (Figure 3). Φ_{PSII} decreased with increasing irradiance, and that decrease was stronger in treated plants than in control plants. The difference in Φ_{PSII} between control and treated plants also increased with time. For black nightshade plants grown in a climate chamber (trial S-1), the difference in Φ_{PSII} between control and treated plants at 4 DAT was significant at irradiance levels above 200 µmol m^{-2} s^{-1}, with reductions of 29, 53, and 66% at 200, 400, and 750 µmol m^{-2} s^{-1}, respectively (Figure 3, S-1). For greenhouse-grown black nightshade plants (trial S-2), reductions in Φ_{PSII} at 4 DAT were largely similar to those found in trial S-1 (15, 48, and 57% at 200, 500, and 700 µmol m^{-2} s^{-1}, respectively) (Figure 3, S-2).

For black nightshade plants grown outside (trial S-3), the differences in Φ_{PSII} between treated and control pants were only 8% (200 µmol m^{-2} s^{-1}) and 32% (750 µmol m^{-2} s^{-1}) at 2 DAT. These differences increased to 16 and 52% (at 200 and 750 µmol m^{-2} s^{-1}, respectively) at 4 DAT and did not increase any further because comparable differences were recorded at 7 DAT (data not shown). Differences in Φ_{PSII} between control and treated plants (at 2, 4, and 7 DAT) measured at light irradiance of 200, 400, and 750 µmol m^{-2} s^{-1} were significant (Figure 3, S-3). For ladysthumb plants grown in a greenhouse (trial P-2), the differences in Φ_{PSII} between treated and control plants were 37 and 42% (at 500 and 700 µmol m^{-2} s^{-1}, respectively) at 2 DAT. This was close to the observations made for black nightshade plants grown under similar circumstances (trial S-2). Significant differences between control and treated plants were observed at light intensities around 500 µmol m^{-2} s^{-1} (Figure 3, P-2).

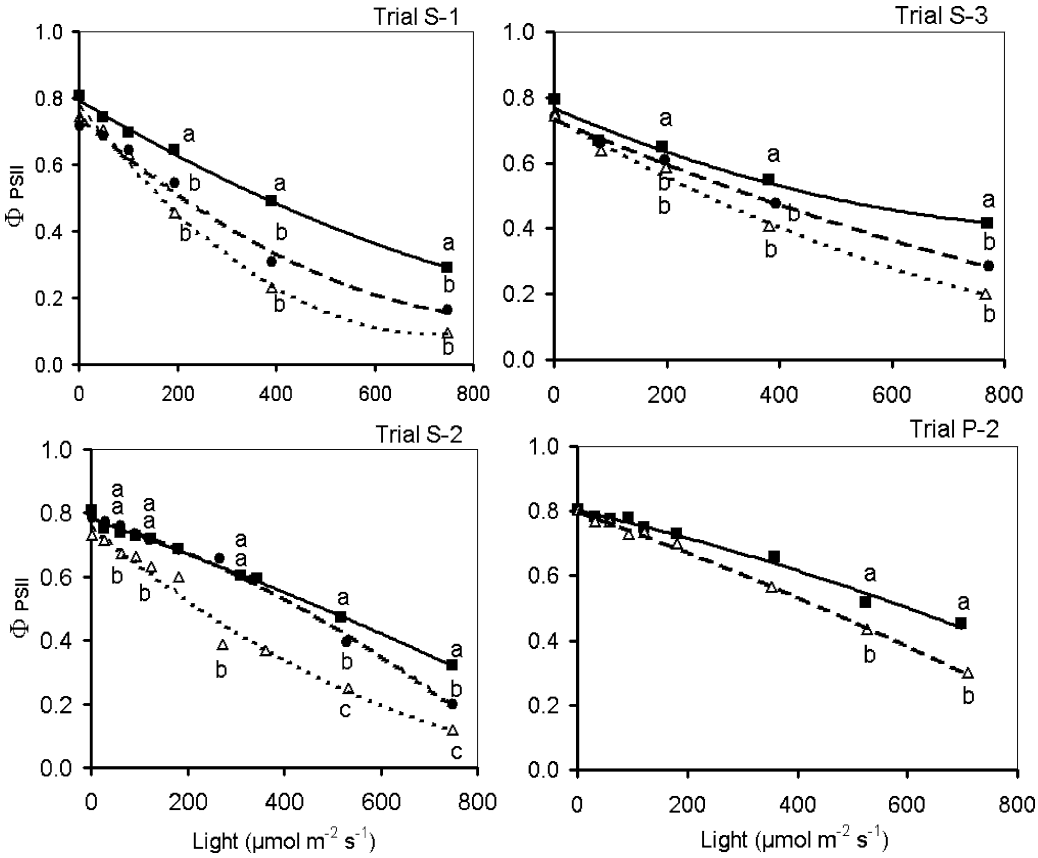

FIGURE 3. Relationship between electron transport through photosystem II (Φ_{PSII}) and irradiance (μmol m^{-2} s^{-1}) for black nightshade plants grown in a climate chamber (S-1), a greenhouse (S-2), and under field conditions (S-3) and ladysthumb plants grown in greenhouse (P-2). Data for control plants (■, solid line) and metsulfuron-treated plants at 2 d after treatment (DAT) (●, dashed line) and 4 DAT (△, dotted line). Treatment measures followed by different letters indicate significant (alpha = 0.05) treatment effects.

At a photon flux density of 0 μmol m^{-2} s^{-1}, Φ_{PSI} was equal to 1.0 for both control and treated plants of black nightshade and ladysthumb at all observation dates. Φ_{PSI} decreased with increasing irradiance. Similar to Φ_{PSII}, this reduction was stronger in treated plants than in the control plants. In all four trials, the trends for the irradiance dependencies of Φ_{PSI} were mostly comparable to those obtained for Φ_{PSII} (Figure 4). For black nightshade plants grown in a climate chamber (trial S-1), the percentage of difference between control and treated plants was comparable to the differences recorded for Φ_{PSII}. Significant differences between control and treated plants were recorded 2 DAT at a light intensity of 400 μmol m^{-2} s^{-1} and higher and at 4 DAT at a light intensity of 80 μmol m^{-2} s^{-1} and higher (Figure 4, S-1). For black nightshade plants grown in greenhouse (trial S-2) or under field conditions (trial S-3), the extent to which Φ_{PSI} decreased with increasing irradiance exacerbated by the herbicide treatment was comparable to the responses described for Φ_{PSII}. In trial S-3, these differences went up to 15 and 43% (at 200 and 750 μmol m^{-2} s^{-1}, respectively) at 4 DAT (Figure 3, S-3) and increased only slightly until 7 DAT (data not shown). For ladysthumb plants grown in greenhouse (trial P-2), the percentage of difference between control and treated plants was comparable to the differences recorded for Φ_{PSII}.

Chlorophyll Content

In treated plants, the level of chlorophyll *a* + *b* was significantly lower than that of control plants (Table 2). Treated black nightshade plants grown in a climate chamber (trial S-1) had their chlorophyll *a* + *b* concentration reduced by approximately 25 and 44% at 2 and 4 DAT, respectively. When grown outside (trial S-3), the reduction in chlorophyll *a* + *b* was comparable (34, 45, and 39% at 2, 4, and 7 DAT, respectively). After herbicide treatment, a slight change in the chlorophyll *a*/*b* ratio was observed for black nightshade plants grown in a climate chamber (trial S-1), but the shift was more dramatic for plants grown in the field (trial S-3). The chlorophyll *a*/*b* ratio of treated black nightshade plants (trial S-3) was 19% (2 DAT) and 30% (7 DAT) lower than that of control plants (Table 2). This ratio indicates that, in the treated plants, the relative loss of chlorophyll *a* was greater than the relative loss in chlorophyll *b*.

Discussion

Herbicide Symptoms and Plant Growth

In the current experiments, the development of clear visual symptoms of metsulfuron toxicity took at least 7 d. A delay of at least 7 d or longer is too long if a second application of herbicide was necessary. From our experience with the development of the MLHD technology for photosynthesis-inhibiting herbicides, it is clear that farmers and agricultural contractors demand an early detection method (within a few days following the application of the herbicides) to assess whether the treatment will be successful in

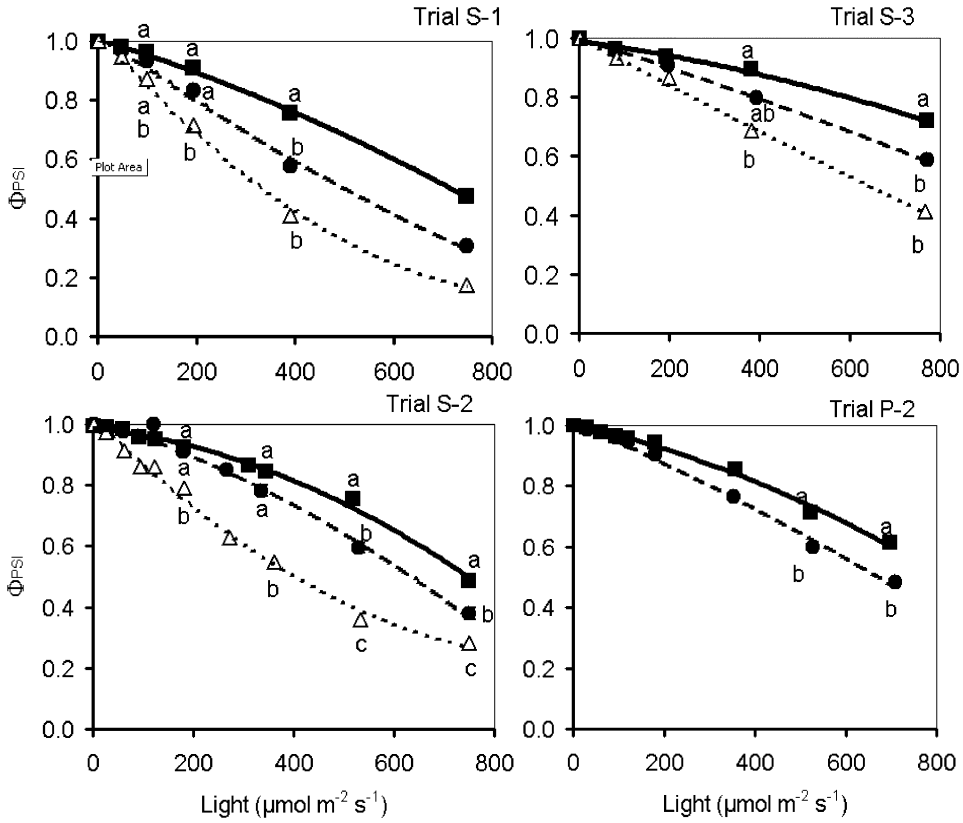

FIGURE 4. Relationship between electron transport through photosystem II (Φ_{PSII}) and irradiance (µmol m^{-2} s^{-1}) for black nightshade plants grown in a climate chamber (S-1), a greenhouse (S-2), and under field conditions (S-3) and ladysthumb plants grown in greenhouse (P-2). Data for control plants (■, solid line) and metsulfuron-treated plants at 2 d after treatment (DAT) (●, dashed line) and 4 DAT (△, dotted line). Treatment measures followed by different letters indicate significant (alpha = 0.05) treatment effects.

eliminating the weeds (Kempenaar et al. 2004). Such a guarantee has contributed to the adoption of the MLHD methodology.

The results were consistent with the observations of Shaner and Singh (1992) that the growth of plants treated with ALS-inhibiting herbicides slows within hours and ceases within a few days, and the findings of Gaston et al. (2003), who observed a clear reduction of plant dry weights at only 7 d after application of the ALS-inhibiting herbicides imazethapyr and chlorsulfuron. Although the assessment of growth by visual or other means is feasible under laboratory conditions, its use under field conditions, possibly on a large scale, would be at best inconvenient and, more generally, unsuitable as a means for the routine assessment of herbicidal toxicity.

Controlled Breakdown or Catastrophic Loss of Photosystem I and II?

The linear relationship between the efficiencies of photosystems I and II (data not shown) suggested a predominant role for linear electron transport—a phenomenon that has been widely reported (Harbinson and Foyer 1991; Harbinson et al. 1990a; Harbinson et al. 1990b; Kingston-Smith et al. 1999). It is noteworthy that the consistent, linear relationship between the efficiencies of both photosystems was mostly sustained under contrasting growing environments and plants species. The linear relationship between the efficiencies of photosystems I and II was unaltered by herbicidal treatment (Figures 3 and 4), even though large changes occurred in the pigment concentration of the leaves

TABLE 2. Cholrophyll concentration (Chl *a+b*) and chorophyll *a/b* ratio for treated and control plants of black nightshade (SOLNI) grown in a climate chamber (CC) or under field conditions (Field). Measurements were taken at 2, 4, and 7 d after treatment (DAT). Treatment measures followed by different letters indicate significant (α = 0.05) treatment effects.

				Chl *a+b*			Chl *a/b*		
Trial	Species	Growth	DAT	Control	Treated	% diff.	Control	Treated	% diff.
				mg g^{-1} fresh weight					
S-1	SOLNI	CC	2	1.705 a	1.272 b	25.4	3.600 a	3.376 a	6.2
S-1	SOLNI	CC	4	1.760 a	0.992 c	43.6	3.526 a	3.364 a	4.6
S-3	SOLNI	Field	2	1.096 a	0.722 b	34.1	3.980 a	3.234 a	18.7
S-3	SOLNI	Field	4	1.069 a	0.586 c	45.2	3.915 a	2.728 b	30.3
S-3	SOLNI	Field	7	0.930 a	0.566 c	39.1	3.966 a	2.822 b	28.8

(Table 2). For field-grown black nightshade plant (trial S-3), however, data suggest that in treated plants the relative loss in Φ_{PSII} was slightly larger than the loss in Φ_{PSI} and that the relative loss of chlorophyll *a* was greater than the loss of chlorophyll *b*. This could be the result of the greater percentage of chlorophyll *b* associated with photosystem II compared with photosystem I. Taken together these data imply that the stress-induced loss of chlorophyll is sufficiently organized in the climate chamber plants (trial S-1) so as to maintain the balance of pigment–protein complexes and function between the photosystems close to that found in control leaves. This loss of leaf chlorophyll in treated plants was not a catastrophic, uncontrolled process but rather the consequence of a well-organized breakdown of components. This situation is similar to that found in cold-grown corn (*Zea mays* L.) 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). Under field conditions (trial S-3), this pattern of controlled breakdown disappears. The greater loss of chlorophyll *a* compared with chlorophyll *b* implies a relatively greater loss of solely chlorophyll *a*–containing chlorophyll–protein complexes, such as the bulk of those associated with photosystem I and the antenna and reaction complexes of photosystem II. The high Φ_{PSII} following dark-adaptation (the measurement at 0 μmol m^{-2} s^{-1}) in herbicide-treated leaves implies an absence of photoinhibition of photosystem II, which rules out a loss of photosystem II activity by that route.

Early Assessment of Herbicidal Effects under Field Conditions

For black nightshade plants grown in the field, a 75% reduction of the CO_2 fixation was observed at 7 DAT, which was 3 d before the first symptoms became noticeable and 2 to 3 wk before plants died. Treated ladysthumb plants also had their CO_2 fixation largely reduced soon after application. Several portable gas analyzers are available for field measurement of gas exchange at leaf level and have been used in a number of field studies (Bernacchi et al. 2002; Myers et al. 1999; Rascher et al. 2000; Tissue et al. 1997). However, portable systems for the measurement of CO_2 fixation are expensive, complicated, and sometimes both, and the time required to make an individual measurement is normally several minutes or longer, depending on the protocol employed. For these practical reasons, we do not believe that measurements of CO_2 fixation are a practical option for field assessment of the activity of metsulfuron on weeds.

The inhibition of Φ_{PSII} that could be detected 2 to 4 d after application of the herbicide proved that Φ_{PSII} in illuminated leaves is a parameter to consider when looking for early assessment tools. Measurements of photosystem II intactness (estimated by means of a dark-adapted Fv/Fm measurement) or steady-state quantum efficiency of photosystem II in illuminated leaves are used to screen for environmental stress tolerance in plant breeding, in air pollution studies (Lichtenthaler and Rinderle 1988; Odasz-Albrigtsen et al. 2000; Popovic et al. 2003), in herbicides toxicity studies (Judy et al. 1991; Percival and Baker 1991), and in environmental stress studies, such as chilling, freezing, heat stress, and nutrient deficiency (De Groot et al. 2003; DeEll and Toivonen 1999; Kingston-Smith et al. 1999; Kingston-Smith et al. 1997). A recent review from Baker and Rosenqvist (2004) exposed the possible applications of chlorophyll fluorescence in crop production. With the development of smaller electronic components and optical systems, instruments for measuring dark-adapted Fv/Fm or Φ_{PSII} have become compact and more readily usable outside the laboratory. Moreover, measurements can be made within a few seconds. Instruments for measuring the kinetics of in vivo fluorescence include the multiflash kinetic fluorometer, the fast repetition rate fluorometer, and the double-modulation fluorometer. Imaging instruments based on charge-coupled device (CCD) cameras have been successful in mapping the photosynthetic activity of a leaf (Bartak et al. 2005; Nedbal et al. 2000; Schreiber et al. 2003). The current results demonstrate that the use of chlorophyll fluorescence to detect the toxicity of ALS-inhibiting herbicides would require the comparison of steady-state Φ_{PSII} for control and treated plants at irradiance around 400 or 500 μmol m^{-2} s^{-1}. When measured at low irradiance (from 0 to 50 μmol m^{-2} s^{-1}), no difference was observed between control and treated black nightshade or ladysthumb plants. Making observations before and after the herbicide treatment would be a more difficult option because the steady-state Φ_{PSII} is affected by any environmental factor that affects CO_2 fixation, such as temperature, irradiance, or drought. This implies that the measurements would need to be made under conditions of controlled irradiance, temperature, and drought, and any other factor that could result in damage to the plant (e.g., frost) would need to be avoided. To produce such a controlled leaf environment and to leave the leaf for sufficient time to allow the Φ_{PSII} to reach a steady-state in response to the treatment would increase the complexity of the equipment and the time required to make the measurement. However, it would still be s simpler, cheaper measurement than a measurement of CO_2 fixation. Another way to deal with the comparison of steady-state Φ_{PSII} measurements between control and treated plants would be to perform the measurements at different locations within the same field. Steady-state Φ_{PSII} of control plants should then be measured in a small patch of the field that is left untreated. The position of such a patch could be randomly chosen or put at a specific spot in the field (e.g., close to the field edge or the start or end of a row).

Measurements of Φ_{PSI} could also detect herbicidal effects. Light-induced absorbance changes at 820 nm (ΔA_{820}), which are used to estimate Φ_{PSI} (Harbinson and Hedley 1993), can also be used to measure the rate constant for electron transport from plastoquinol through the cytochrome b6/f complex to $P700^+$, which in turn can be used to provide an estimate of changes in the light-saturated rate of CO_2 fixation. As light-saturated rates of CO_2 fixation decrease following treatment with ALS-inhibiting herbicides, the application of the ΔA_{820} measurement could be used as an indicator for herbicide activity. However, for this application to work, the chlorophyll content of the leaf must not change, and in this and previous studies (Riethmuller-Haage et al. 2006), changes of chlorophyll content have been observed. As the maximum ΔA_{820} was also shown to decrease following the application of metsulfuron, measur-

ing the maximum ΔA_{820} (i.e., that obtained under far-red illumination), which is proportional to the amount of P700, would be an alternative approach. Changes in ΔA_{820} are easy and rapid to assess (typically only requiring less than 10s). However, as changes in P700 were broadly parallel to those of chlorophyll content, it might be even easier to evaluate the loss in leaf chlorophyll content using readily available portable chlorophyll meters. These can produce a measurement of leaf chlorophyll content within 5 to 10 s and, based on the measurements of chlorophyll presented here, would likely be a useful method for identifying the effectiveness of a metsulfuron treatment.

Our results demonstrated that the different parameters measured showed large differences between control and treated weed plants soon after application of the ALS-inhibiting herbicides metsulfuron, and in principle, all of them could be used as an early indication for a successful application. The practical applicability, however, actually determines which parameter is best suited for field assessment of herbicide efficacy. Measurement of the quantum efficiency of Φ_{PSII} and the loss in leaf chlorophyll content appeared to be two of the most practical parameters to use when designing an early detection method for assessing the toxicity of metsulfuron. The use of chlorophyll fluorescence would require a comparison of steady-state Φ_{PSII} measurements for control and treated plants, which could be realized either by measuring in time (before and after application) or space (treated or untreated patch).

Sources of Materials

[1] TL-D-HF lamps, Philips, High Tech Campus 34, P.O. Box WB 01 5656 AE, Eindhoven, The Netherlands.

[2] Mark 3, Analytical Development Company, Hertford Road, Hoddesdon, EN11 9BU, UK.

[3] Calfex dichroic mirrors, Unaxis Balzers Ltd., P.O. Box 1000, 9496 Balzers, Liechtenstein.

[4] Metal film neutral-density filters, Unaxis Balzers Ltd., P.O. Box 1000, 9496 Balzers, Liechtenstein.

[5] Shimadzu UV 160-A, Shimadzu Scientific Instruments, 7102 Riverwood Drive, Columbia, MD 21046.

[6] Genstat 7.2, Lawes Agricultural Trust, Institute of Arable Crops Research-Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, Great Britain.

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