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# Predicting herbicidal plant mortality with mobile photosynthesis meters

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## Summary

Herbicide dose optimisation, i.e. maximising weed control and crop yield with herbicide dose, is an important part of integrated weed management strategies. However, the adoption of optimised dose technology and variable rate application has been limited because of the relatively long period between herbicide treatment and the time when efficacy can be visually assessed. Herbicide dose optimisation could therefore benefit from simple methods that allow early prediction of plant mortality. Early prediction would allow better management decisions, e.g. timely retreatment in case of uncontrolled weeds. The focus of this study was the relationship between leaf photosynthesis soon after herbicide treatment and subsequent plant mortality, with the aim of determining whether the former could predict the latter. Data from 28 glasshouse experiments were analysed. In these experiments, herbicides from five modes of action groups were tested on five plant species. Leaf photosynthesis was measured with two mobile meters up to 1 week after herbicide treatment. Leaf photosynthesis was affected by plant species, leaf number, herbicide species, dose and time. Large changes in leaf photosynthesis were observed with photosynthesis-inhibiting herbicides, intermediate changes were noted with glyphosate, glufosinate-ammonium and sulcotrione, and no changes were detected with MCPA. Threshold values associated with plant mortality were then determined. These values can be used to assess the risk of uncontrolled weeds treated with variable herbicide doses.

**Keywords:** herbicide efficacy, MLHD, dose optimisation, photosynthesis, decision support system, sustainable herbicide use.

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## Introduction

Modern agriculture largely depends on herbicides for weed control. Concerns about herbicide residues in our environment, food and drinking water (Lotz *et al*., 2002; Bannink, 2004; Kempenaar *et al*., 2007) require farmers to minimise herbicide use whenever possible. Other incentives for farmers to do this are savings on herbicide costs and reducing herbicide crop injury. Minimising herbicide use can be partially achieved by reducing the dose. However, reducing herbicide doses can result in a higher risk of uncontrolled weeds and may favour the evolution of herbicide resistance in weeds (Gressel, 1995; Powles *et al*., 1998a,b; Neve, 2007). Optimisation of herbicide dose is therefore necessary to improve the sustainability of agricultural production systems (Kudsk & Streibig, 2003; Rüegg *et al*., 2007; Kropff *et al*., 2008).

Farmers have adopted various strategies to optimise herbicide dose, such as split application or low dose systems. How much the recommended dose (found on

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the label) can be reduced while still being effective is determined by complex interactions between many factors, such as weed species, weed stage, crop stage, weather and soil conditions, spray technology, herbicide formulation and economics (e.g. Kudsk & Kristensen, 1997). Decision support systems (DSS), which provide guidelines for reduced doses by taking these interactions into account, have become available (e.g. Kudsk, 1999; Kempenaar *et al*., 2002) and are essential for sustainable optimisation of herbicide dose (Christensen *et al*., 2009; Rydahl, 2009). A limiting factor for the adoption of reduced doses is the time before efficacy can be determined with certainty. On average, this period is 3–4 weeks (Powles *et al*., 1998a,b; Hashem *et al*., 2001; Van Eerd *et al*., 2005). Shortening this period could facilitate the adoption of reduced doses and DSS.

Ketel *et al*. (1996; 1997) proposed a model for dose optimisation of metribuzin using a mobile fluorescence meter to monitor efficacy. Christensen *et al*. (2003) and Streibig *et al*. (2008) used a fluorescence meter to study the effects of herbicides on plants. Others (e.g. Van Oorschot, 1970; Duke, 1985; Van Rensen, 1988; De Ruiter *et al*., 2005, 2007; Riethmuller Haage *et al*., 2006) used larger, immobile indoor photosynthesis research setups for this purpose. Still lacking, however, is an extensive evaluation of the relationship between leaf photosynthesis – measured with mobile meters soon after herbicide application – and plant mortality.

This paper presents the results of a study on the relationship between leaf photosynthesis and plant mortality under controlled glasshouse conditions. Leaf photosynthesis was measured with mobile meters during the first week after herbicide treatment. Herbicides with five different modes of action and several plant species were studied. The research objectives were as follows:

(i) to describe the dynamics of leaf photosynthesis after herbicide treatment,
(ii) to evaluate whether herbicide plant mortality could be predicted with mobile leaf photosynthesis meters and
(iii) to determine what minimum photosynthesis activity values were associated with plant mortality.

## Materials and methods

Twenty-eight glasshouse experiments were conducted between 1999 and 2006. In each experiment, 18–40 plants were treated using differing doses of a herbicide or herbicide mixture. Various plant responses (including two leaf photosynthesis parameters, visible herbicide damage and plant mortality) were then assessed, for up to 1 month after treatment. Each experiment had a completely randomised design with 4–8 replicates per treatment.

### *Plant material and growth conditions*

The glasshouse was located in Wageningen, the Netherlands (51°59′11N, 05°39′52E). Plants were grown in the glasshouse in 1 L pots containing a mixture of peat potting soil and coarse sand (2:1 wt/wt). Water and nutrients were adequately supplied. Day temperature was set at 18°C, night temperature at 12°C and relative humidity at 70%. Photoperiod and light intensity were influenced by season. The photoperiod was at least 12 h and was provided by natural light supplemented with high-pressure mercury lamps.

Plant species included weed versus crop and grass versus broadleaved species: *Chenopodium album* L., *Echinochloa crus-galli* L., *Solanum nigrum* L., *Spinacia oleracea* L. and *Triticum aestivum* L. Seeds were collected from mature plants at least 1 year prior to the experiments. Initially, several seeds were planted, but seedlings were thinned to one per pot. Plant stage was assessed at time of treatment by counting the true leaves with a length $>1$ cm. Plants had 3–6 true leaves at the time of treatment (BBCH code 13–16; Anonymous, 1994).

### *Herbicide application*

Herbicides were applied as single post-emergence applications using an air-pressurised laboratory track sprayer (1 m $s^{-1}$) delivering 400 L $ha^{-1}$ (Birchmeier 1.2-mm cone nozzles, 300 kPa, medium fine droplets). The following herbicides were used: bentazone + terbuthylazine (Laddok N, 200 + 200 g a.i. $L^{-1}$, SC; BASF, Arnhem, the Netherlands), metamitron (Goltix WG, 700 g a.i. $kg^{-1}$, WG; Bayer, Mijdrecht, the Netherlands), metoxuron (Dosanex, 800 g a.i. $kg^{-1}$, EC; Cyanamid Agro, Breda, the Netherlands), metribuzin (Sencor WG, 700 g a.i. $kg^{-1}$, WG; Bayer), phenmedipham (Agrichem Fenmedifam, 157 g a.i. $L^{-1}$, EC; Agrichem, Oosterhout, the Netherlands), glyphosate (Roundup Evolution, 360 g a.i. $L^{-1}$, SL; Monsanto Europe, Brussels, Belgium), glufosinate-ammonium (Finale SL 14, 150 g a.i. $L^{-1}$, SL; Bayer), MCPA (U 46 MCPA, 500 g a.i. $L^{-1}$, SL; BASF) and sulcotrione (Mikado, 300 g a.i. $L^{-1}$, SC; Bayer). Only the first four herbicide formulations listed are photosynthesis-inhibiting herbicides (PS inhibitors) (http://www.hracglobal.com; 27 August 2010).

### *Observations and photosynthesis measurements*

Plant responses to herbicide treatments were periodically assessed for up to 1 month after treatment. Leaf

photosynthesis parameters were measured with two mobile meters: the Plant Photosynthesis Meter (PPM) and the PhotoSystem 1 Meter (PS1).

The PPM measures photosystem II activity. A PPM-200 model from the EARS Company (Delft, the Netherlands) was used. A PPM reading measures photosynthetic efficiency ($\Phi$), where $\Phi = (1 - F/F_m) \times 100\%$ and $F$ = fluorescence under dark conditions and $F_m$ = fluorescence under saturated light conditions. PPM readings are given on a scale from 80 (optimal photosynthetic efficiency) to 0 (no photosynthetic efficiency). The PPM projects 660 nm wavelength light onto the study surface and measures the fluorescence reflection. The measurements were taken non-destructively on the upper sides of dark-adapted leaves (30-min adaptation), using the youngest measurable leaves $>2$ cm$^2$ (in one experiment, all the leaves were measured). This was carried out on about 1 cm$^2$ of leaf area at the centres of the leaves, immediately prior to treatment and for up to 1 week after treatment (WAT).

The PS1, made by the Rometron Company (Doorwerth, the Netherlands), measures photosystem I activity. Leaves were placed in the clip of the PS1 just before the measurement was taken. Light absorbance of the leaf tissue at 820 nm was then measured. The timing and focal point of the measurements with the PS1 were similar to those of the PPM. Readings of the PS1 meter are given on a scale from 0 (no damage to photosystem I) to 100% (total block of electron transport).

For more background information on the PPM and PS1 meters, see Annex S1, Genty & Harbinson (1996), Kempenaar (2004), Kempenaar & van den Boogaard (2004) and, http://www.mlhd.nl and http://www.ears.nl/ppm. Annex S2 contains additional information on whole plant leaf photosynthesis measurements.

### *Statistical analyses*

Leaf photosynthesis data were analysed by ANOVA to determine herbicide and date of observation effects using GenStat (12th release, Payne *et al.*, 2009). Fisher's $LSD_{0.05}$ was calculated to determine significant differences between treatment means. The correlation between leaf photosynthesis measured with the PPM and PS1 meters was determined using linear regression.

The correlation between leaf photosynthesis soon after herbicide treatment and plant response (a binomial parameter, plants were either dead or alive at the end of the experiment) later on was analysed using frequency distribution plots and, where possible, non-linear logistic regression of the GenStat program. Leaf photosynthesis frequency distributions were plotted for the plants that were killed by the herbicide treatment and those plants that survived the herbicide treatment to determine whether the frequency distributions distinguished the groups. If the overlap of curves was small, the leaf photosynthesis data were fitted to the logistic model:

$$Y = a + (c - a)/(1 + \exp(-b \times (X - m))) \qquad (1)$$

where $Y$ corresponds with the plant response (%), $X$ is the leaf photosynthesis parameter measured with the PPM or PS1 meter 2 or 3 days after treatment (DAT), $a$ and $c$ are the lower and upper response limits (asymptotes) of the curve of the model, $m$ is the median location parameter of the curve and $b$ is the slope of the curve at the median. Leaf photosynthesis parameters 2 or 3 DAT that predict plant mortality with a high probability were estimated with the regression model. Two-tailed Student's $t$-tests were carried out to determine significant ($P < 0.05$) differences between parameter estimates of the logistic curves.

## Results

### *Leaf photosynthesis dynamics*

The photosynthesis dynamics of the four-leaf stage *S. nigrum* was affected by metribuzin soon after the herbicide treatment [Fig. 1; significant interactions between herbicide dose and date of observation ($P < 0.01$)]. The PPM readings for untreated plants were between 70 and 80, and the PS1 readings were between 0 and 15. The leaves of plants treated with a high dose (140 g of metribuzin ha$^{-1}$) showed a faster and larger decline in leaf photosynthesis than those treated with a low dose (28 g of metribuzin ha$^{-1}$). Maximum decline was observed at 2 DAT. At 4 and 6 DAT, leaf photosynthesis remained low for the high dose treatment, but partially recovered at low doses. Measurements ceased after 1 week because of leaf necrosis. Plants treated with the high dose died at 3 weeks after treatment (WAT). Plants treated with the low dose, however, were alive and vital at 3 WAT.

When the leaves of *S. nigrum* plants were older and more mature, leaf photosynthesis was less affected by the metribuzin treatment (Fig. 2). Leaf number had a significant effect ($P < 0.01$) on photosynthesis, while observation date was not significant ($P > 0.05$). The plants in this experiment were alive and vital at 3 WAT. There was a good correlation between PPM and PS1 in the experiments with *S. nigrum* and metribuzin (Figs 1 and 2). PS1 $= -1.24 \times$ PPM $+ 95.5$ ($P < 0.001$ and $R^2 = 0.94$).

The leaf photosynthesis dynamics of other combinations of plants and photosynthesis-inhibiting herbicides were affected by the susceptibility of the plant species to the herbicide [Fig. 3, significant interactions ($P < 0.05$)

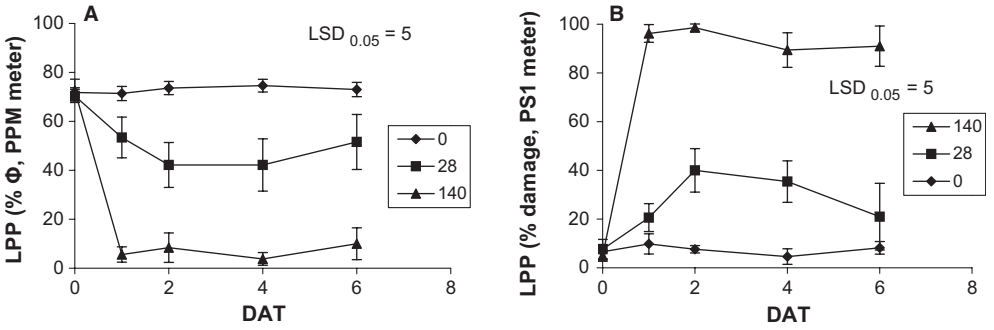


**Fig. 1** Leaf photosynthesis parameter (LPP) of 3- to 4-leaf *Solanum nigrum* plants treated with metribuzin (0, 28 or 140 g of metribuzin $ha^{-1}$) measured with Plant Photosynthesis (A) and PhotoSystem 1 meter (B). Treatment means ($n = 5$) and standard deviations (error bars) are shown. DAT is days after herbicide treatment.

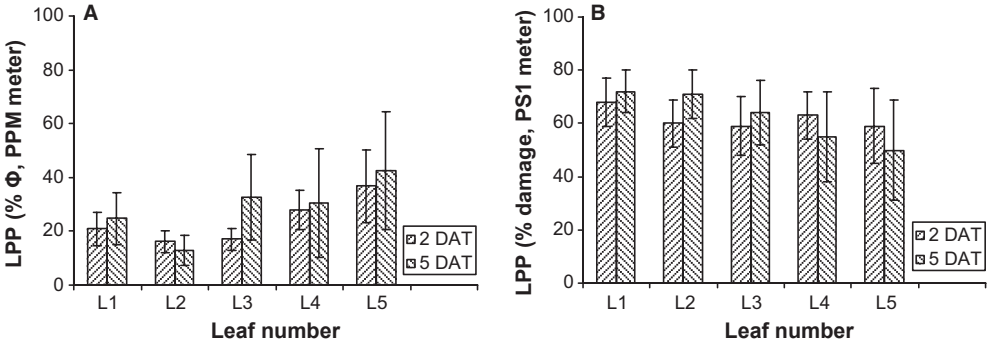


**Fig. 2** Leaf photosynthesis parameter (LPP) of leaf 1–5 of six-leaf stage *Solanum nigrum* plants treated with 140 g of metribuzin $ha^{-1}$ measured with Plant Photosynthesis (A) and PhotoSystem 1 meter (B). Treatment means (8 replicates) and standard deviations (error bars) are shown. $LSD_{0.05} = 7.8$ (A) or 15.5 (B). DAT is days after herbicide treatment.

between herbicide dose and date of observation in all experiments]. Whether plants died from a herbicide dose at 3–4 WAT is reported in the legend for Fig. 3. Changes in leaf photosynthesis for the combinations *S. nigrum* × metamitron, *E. crus-galli* × bentazone + terbuthylazine, *C. album* × metoxuron and *C. album* × phenmedipham (Fig. 3A,C,D and F) were comparable with those of *S. nigrum* × metribuzin (Fig. 1A). They represent cases of plant–herbicide combinations where the plant is known to be sensitive to the herbicide with a post-emergence treatment. In these cases, plants treated with a lethal dose showed a faster and larger decline in leaf photosynthesis than plants treated with a sublethal dose during the first 2–3 DAT, and plants treated with a sublethal dose showed recovery of leaf photosynthesis from 4 DAT onwards. However, *C. album* × metamitron and *S. oleracea* × phenmedipham showed a smaller decline in leaf photosynthesis (Fig. 3B and E). A small, dose-dependent effect on photosynthesis activity was observed up to 4 DAT, and the plants did not die from the herbicide doses. They represent cases of plant–herbicide combinations where the plants are known to be slightly sensitive or not sensitive to the herbicide with a post-emergence treatment.

Leaf photosynthesis was also affected by several non-photosynthesis-inhibiting herbicides during the first WAT (Fig. 4). Whether plants had died from the herbicide dose at 3–4 WAT is reported in the legend for Fig. 4. Glufosinate-ammonium, sulcotrione and glyphosate caused a dose-dependent decline in leaf photosynthesis (only PPM data are shown, but PS1 were similar) during that week. The decline peaked at about 4–7 DAT, which was later than for the photosynthesis-inhibiting herbicides. Significant differences between treatments were measured with the PPM and PS1 meters at 3 DAT onwards. MCPA did not affect leaf photosynthesis during the first WAT, while the high MCPA doses were lethal to the plants at the end of the experiment.

### *Plant mortality*

Frequency distribution plots were used to determine whether photosynthesis measurements soon after herbicide treatment were correlated with plant mortality at the end of the experiment (Fig. 5 and 6). When the plots showed that the frequency distribution of plants that survived the herbicide treatment only slightly

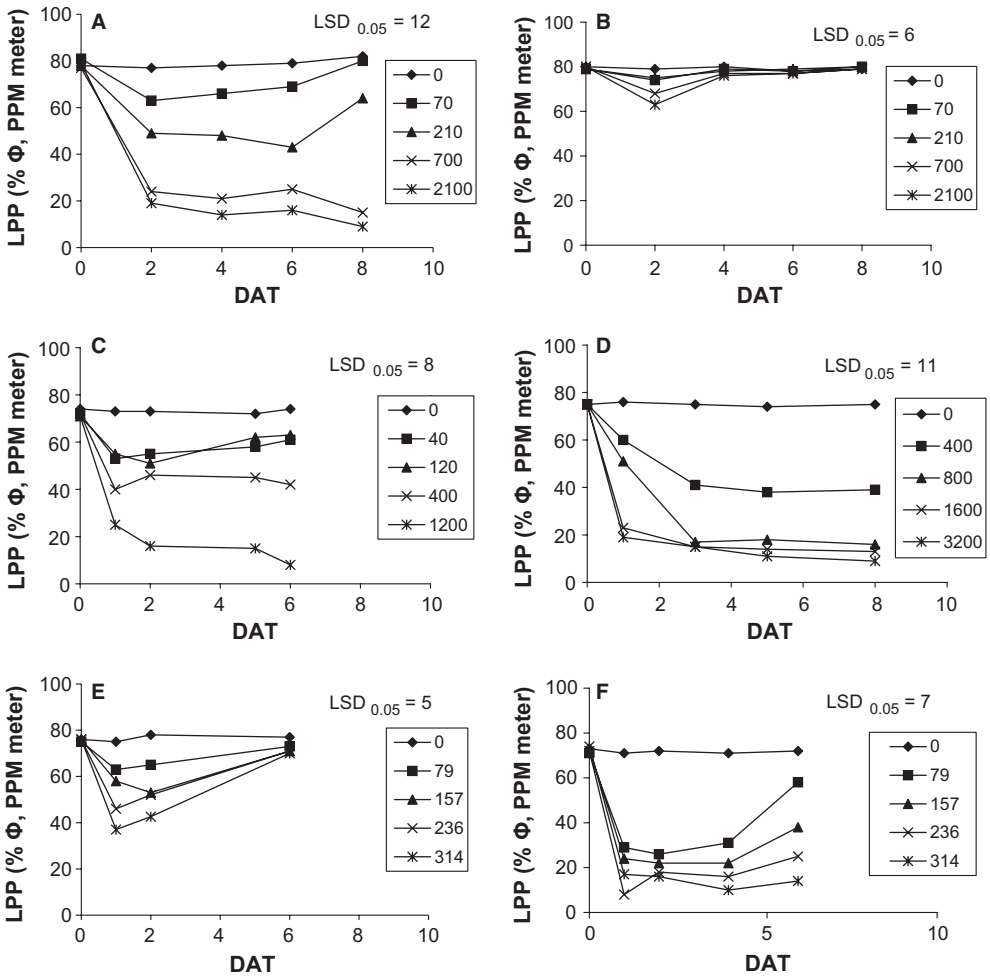


**Fig. 3** Leaf photosynthesis parameters (LPP) of plants treated with photosynthesis inhibiting herbicides. Each subfigure legend shows herbicide dose [g a.i. $ha^{-1}$]. (A) Metamitron and four-leaf *Solanum nigrum*, 700 and 2100 g a.i. $ha^{-1}$ were lethal treatments. (B) Metamitron and four-leaf *Chenopodium album*, no lethal treatments. (C) Bentazone + terbuthylazine and four-leaf *Echinochloa crus-galli*, 1200 g a.i. $ha^{-1}$ were a lethal treatment. (D) Metoxuron and five-leaf *C. album*, 800, 1600 and 3200 g a.i. $ha^{-1}$ were lethal treatments. (E) Phenmedipham and three-leaf *Spinacia oleracea*, no lethal treatments. (F) Phenmedipham and four-leaf *C. album*, 314 g a.i. $ha^{-1}$ were a lethal treatment. Treatment means (four or five replicates) and $LSD_{0.05}$ are shown.

overlapped the distribution of plants that died from the herbicide treatment, additional regression analysis was carried out to assess photosynthesis values associated with plant mortality. Data from individual experiments were combined for each herbicide mode of action group (generally >100 plants per group).

The frequency distributions distinguished plants that died from or survived a photosynthesis-inhibiting herbicide dose (Fig. 5A and B). Figure 5A (based on the PPM) shows the frequency distribution plot of 160 individual plants treated with a photosynthesis-inhibiting herbicide in eight experiments (five plant species, five herbicides and doses as indicated in Figs 1–3). Dead plants in Fig. 5A appear primarily on the left side of the *x*-axis, while plants that survived the treatment appear in the middle and on the right side of the *x*-axis. The overlap zone of the distributions was around PPM = 20. Of the plants that died from the herbicide treatment, 87% had readings of PPM ≤ 20 2 DAT, and 98% of the plants that survived the treatment had readings of PPM > 20.

Figure 5B (based on the PS1) shows the frequency distribution plot of 194 individual plants treated with a photosynthesis-inhibiting herbicide in eight experiments (five plant species, five herbicides, and doses as indicated in Fig. 1–3). Dead plants in Fig. 5B are mainly seen on the right side of the *x*-axis, while plants that survived the treatment are mainly in the middle and on left side of the *x*-axis. The overlap zone of the distributions was around PS1 = 65. Of the plants that died from the herbicide treatment, 93% had readings of PS1 > 65 2 DAT; 96% of the plants that survived the treatment had PS1 ≤ 65.

Frequency distribution plots for four non-photosynthesis inhibiting herbicides 3 DAT (Fig. 6) showed more

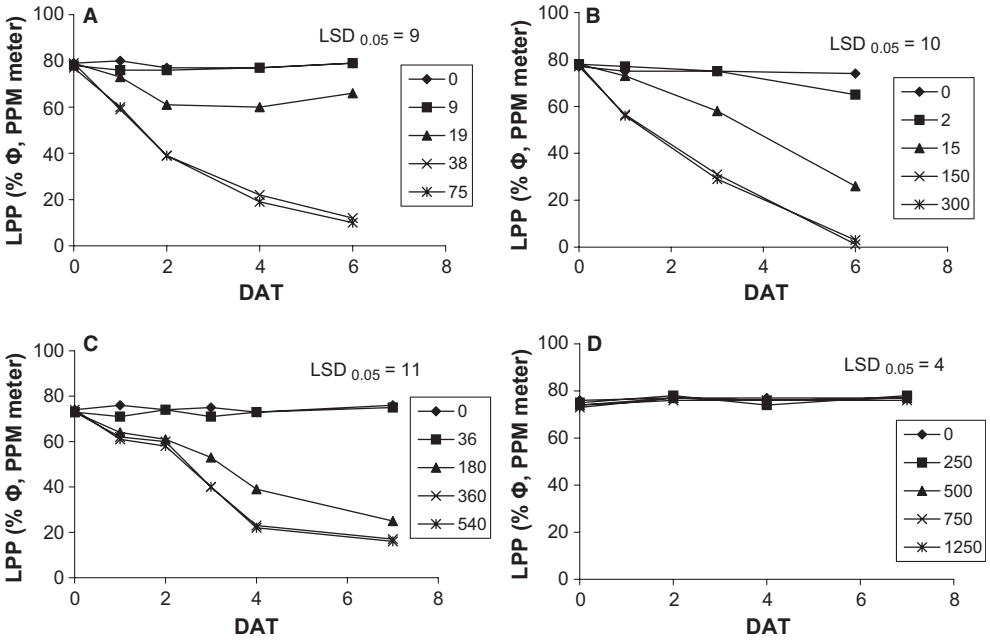


**Fig. 4** Leaf photosynthesis parameters (LPP) of plants treated with non-photosynthesis-inhibiting herbicides. Legend is herbicide dose [g a.i. $ha^{-1}$]. (A) Glufosinate ammonium, 38 and 75 g a.i. $ha^{-1}$ were lethal treatments. (B) Sulcotrione, 150 and 300 g a.i. $ha^{-1}$ were lethal treatments, 15 g a.i. $ha^{-1}$ was lethal to some plants. (C) Glyphosate, 180, 360 and 540 g a.i. $ha^{-1}$ were lethal treatments. (D) MCPA, 1250 g a.i. $ha^{-1}$ was a lethal treatment, 750 and 1000 g a.i. $ha^{-1}$ were lethal to some plants. Treatment means (four or five replicates) and $LSD_{0.05}$ are shown.

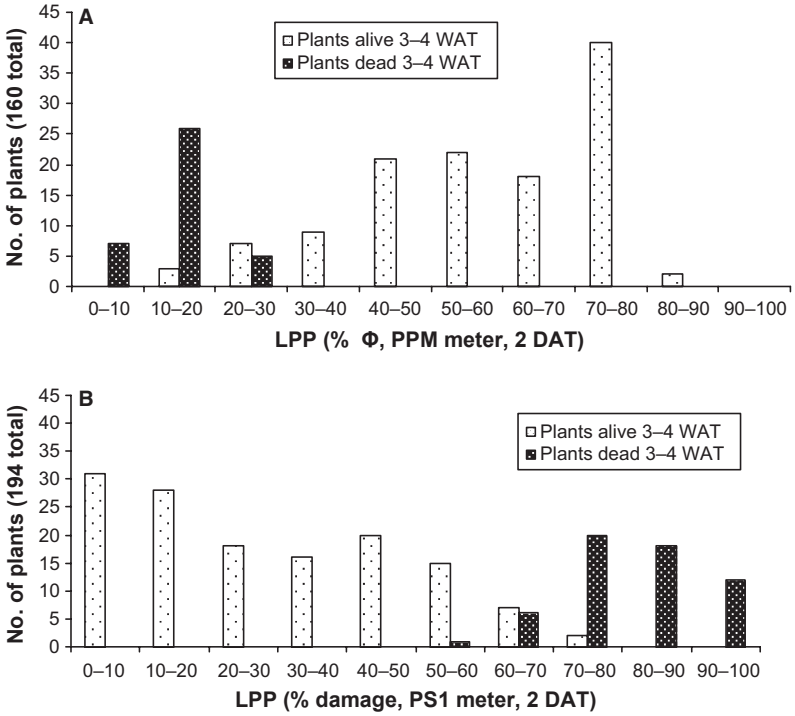


**Fig. 5** Frequency distributions of leaf photosynthesis parameters (LPP) and plant response (dead or alive) 3–4 weeks after treatment for four plant species and five photosynthesis-inhibiting herbicides. LPP was measured with Plant Photosynthesis Meter (A) or PhotoSystem 1 (B) 2 days after treatment.

overlap than those for photosynthesis-inhibiting herbicides at 2 DAT. The data sets of the non-photosynthesis-inhibiting herbicides consisted of 80–100 individual plants, two species and doses indicated in Fig. 4. For glufosinate-ammonium, sulcotrione and glyphosate, the overlap of the distributions of plants that survived and plants that died still allowed an in-depth analysis (Fig. 6A–6C). The overlap zones ranged from 30 to 70 PPM. When PPM ≤ 40 at 3 DAT, plants were dead at the end of the experiments in 95% of the cases. For

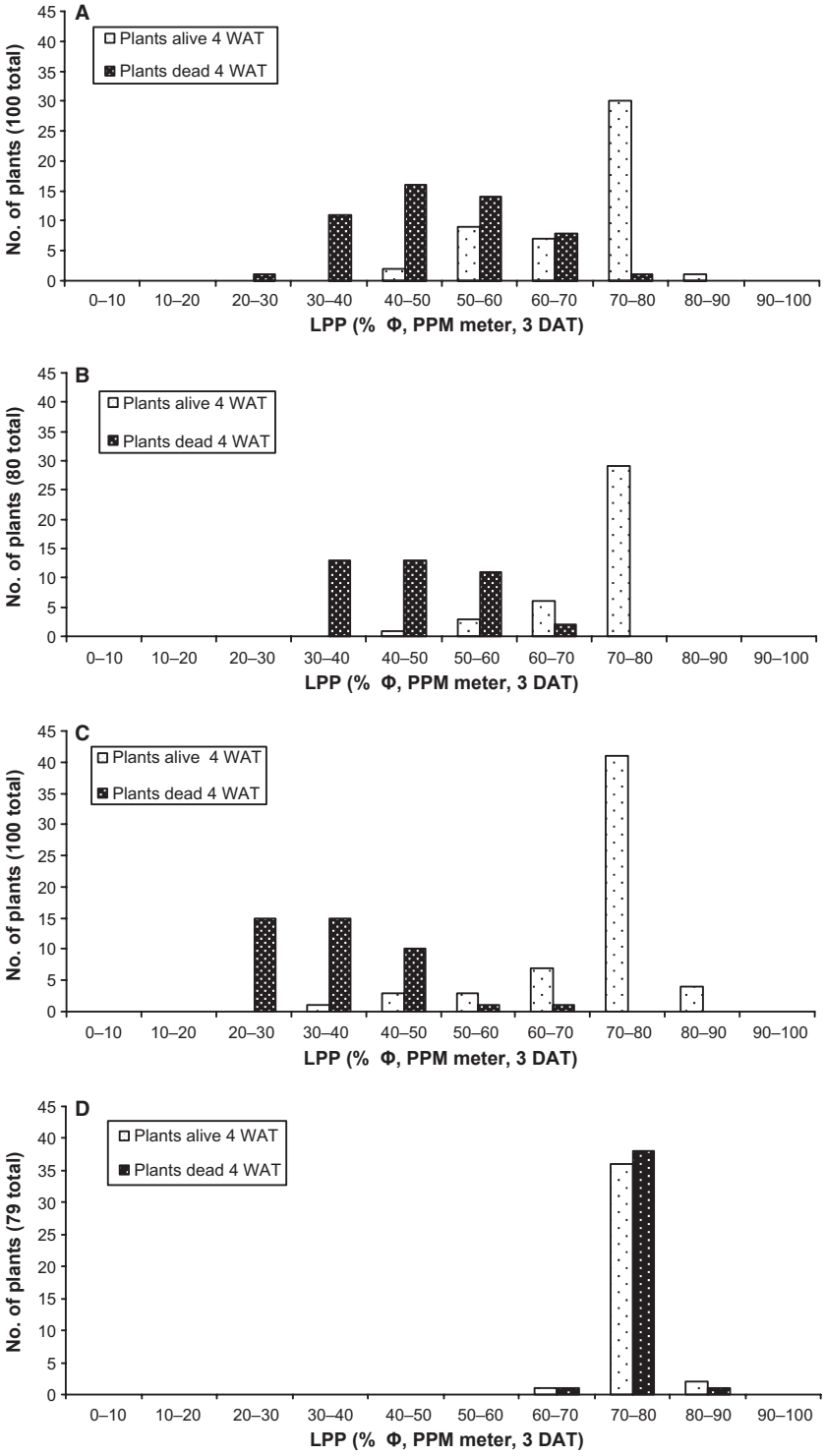


**Fig. 6** Frequency distributions of leaf photosynthesis parameter (LPP) measured with Plant Photosynthesis Meter 3 days after treatment and plant response (dead or alive) 4 weeks after treatment for *Solanum nigrum* and *Chenopodium album* plants. (A) Glufosinate ammonium. (B) Sulcotrione. (C) Glyphosate. (D) MCPA.

PS1, the threshold value that predicted plant mortality was PS1 > 45 at 3 DAT. In the case of MCPA, frequency distributions of plants that survived the herbicide did not distinguish those plants that died from the herbicide Fig. 6D).

The relationship between leaf photosynthesis shortly after treatment and plant response at the end of the experiment was further analysed, using regression for the group of photosynthesis-inhibiting herbicides and for glufosinate-ammonium, sulcotrione and glyphosate

**Table 1** Parameter values ± SE of a logistic model* fitted to data of leaf photosynthesis of individual plants soon after herbicide treatment and plant response (% plants alive) 3–4 weeks after treatment. Data of experiments were combined per herbicide group (PS = photosynthesis). Leaf photosynthesis was measured 2 or 3 days after treatment (DAT) with Plant Photosynthesis Meter (PPM) or PhotoSystem 1 (PS1) meter. $LPP_{90\%}$ is leaf photosynthesis parameter associated with 90% plant mortality according to the model

| Herbicide | Measurement | *m* | *b* | $LPP_{90\%}$ |
|---|---|---|---|---|
| PS inhibitors | PPM 2 DAT | 23.5** ± 1.8 | 0.37 ± 0.13 | 17.6** ± 1.9 |
| Glyphosate | PPM 3 DAT | 50.3 ± 2.8 | 0.20 ± 0.06 | 40.1 ± 3.5 |
| Sulcotrione | PPM 3 DAT | 58.5 ± 2.2 | 0.26 ± 0.08 | 50.0 ± 2.9 |
| Glufosinate-ammonium | PPM 3 DAT | 59.4 ± 2.0 | 0.17 ± 0.04 | 46.6 ± 3.6 |
| PS inhibitors | PS1 2 DAT | 68.1 ± 1.7 | −0.34 ± 0.11 | 74.6 ± 2.4 |

*Logistic equation: $Y = a + (c - a)/(1 + \exp(-b \times (X - m)))$.
**66.3 and 73.6, respectively, according to $PS1 = -1.24 \times PPM + 95.5$.

individually. The logistic model was fitted to the data of Figs 5A, B and 6A–C. The results of the fits are shown in Table 1 (parameters) and Fig. 7 (curves). The standard errors of the estimated model parameters were relatively small (Table 1), indicating that the model fitted well to the data. Differences between parameter estimates were determined with Student's *t*-tests ($P < 0.05$). The location (*m*) parameter and slope (*b*) of the curve of photosynthesis-inhibiting herbicides and PPM 2 DAT were significantly different from those of the glyphosate, sulcotrione and glufosinate-ammonium curves. For the three non-photosynthesis-inhibiting herbicides, differences between the parameters were small. Only the *m* parameters for glyphosate, glufosinate-ammonium and glyphosate and sulcotrione were significantly different. The *b* parameters were not significantly different for these three herbicides. When the PPM parameters for photosynthesis-inhibiting herbicides in Table 1 were converted into PS1 parameters, using the regression equation $PS1 = -1.24 \times PPM + 95.5$, the parameters were not significantly different. Leaf photosynthesis parameters ($LPP_{90\%}$) associated with 90% plant mortality, calculated with the logistic regression equations (Table 1), differed only a few scale points from leaf photosynthesis values associated with plant mortality derived from the frequency distribution plots (Figs 5 and 6).

## Discussion

This study shows that changes in leaf photosynthesis of plants can be measured during the first week after herbicide treatment using mobile meters for herbicides from four of the five modes of action. The changes were dependent on herbicide species, dose, plant species, leaf number and time. The largest and fastest changes in leaf photosynthesis were observed with photosynthesis-inhibiting herbicides, and intermediate changes were observed with glyphosate, glufosinate-ammonium and sulcotrione, while no changes were observed with MCPA. With this knowledge of the dynamics of leaf photosynthesis over time, leaf photosynthesis values associated with plant mortality can be determined to set threshold values predicting plant mortality for different herbicide modes of action.

Leaf photosynthesis values associated with plant mortality were mainly influenced by herbicide (or herbicide group) and date of observation, and only slightly or not at all by plant species. If PPM readings of the youngest measurable leaves of plants treated with a photosynthesis-inhibiting herbicide were <15 or PS1 readings >80 2–5 DAT, nearly all plants (>95%) died from the treatment. In contrast, if PPM readings were >30 or PS1 < 60, 2–5 DAT, then the plants survived the herbicide treatment.

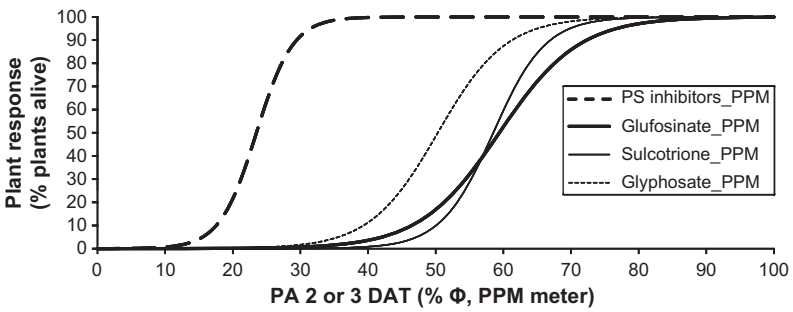


**Fig. 7** Relationship between leaf photosynthesis soon after treatment (2–3 days after treatment) and plant response 3–4 weeks after treatment for different herbicide groups with Plant Photosynthesis Meter. See Table 1 for explanation of regression model and legend.

Glufosinate-ammonium, glyphosate and sulcotrione also affected leaf photosynthesis shortly after treatment. If PPM readings of the youngest measurable leaves of treated plants were < 40 or PS1 readings > 45 3–5 DAT, nearly all plants died. The primary mode of action of these herbicides is inhibition of specific proteins or enzymes. This inhibition causes a measurable decline in leaf photosynthesis during the first week of treatment. However, in contrast with photosynthesis-inhibiting herbicides, there is a range of readings which are inconclusive. If PPM readings were between 40 and 70 or PS1 readings were between 20 and 45 3–5 DAT, then the readings could not be used to predict whether the plants would die from the glufosinate-ammonium, glyphosate or sulcotrione treatments.

Leaf photosynthesis values were not associated with herbicidal plant mortality in the case of MCPA. This is not surprising, considering the mode of action of this herbicide. MCPA is a synthetic auxin stimulating local cell growth and is not likely to affect photosynthesis during the first DAT.

The aforementioned threshold values apply to young plants (up to six-leaf stage) grown from seeds. In cases of larger annual plants or perennial plants, more variation in leaf photosynthesis and less correlation between leaf photosynthesis and herbicidal plant mortality have been observed.

In this study, the focus was on herbicidal plant mortality, because weed kill is the objective of weed control strategies, rather than weed growth reduction. In recent years, good correlations between leaf photosynthesis and growth reduction of plants treated with sublethal doses of photosynthesis-inhibiting herbicides and acetolactate synthase-inhibiting herbicides have been demonstrated by Ketel *et al.* (1996), Christensen *et al.* (2003), Kempenaar & van den Boogaard (2004), Riethmuller Haage *et al.* (2006) and Zhang *et al.* (2006).

This study involved 5 of the 16 known herbicide modes of action of commercial herbicides. Additional research is required to determine the full potential of leaf photosynthesis meters for predicting herbicidal plant mortality. It is assumed that it will be possible to use these meters for about 50% of the known modes of action. This study has shown that mobile photosynthesis meters have a good potential for the early prediction of herbicidal plant mortality and herbicide dose optimisation in agriculture. The need for additional treatment could be assessed with this approach. If users understand how to use the leaf photosynthesis meters, how to minimise the variation in readings and how to interpret values associated with plant mortality, they could make better decisions on herbicide use and dose. This could, for example, be beneficial in a situation where rain occurs shortly after a herbicide application. Leaf photosynthesis measurements on key weeds in the field could then provide information on the need for an additional treatment, and the farmer could act accordingly. The measurements would take approximately 1–2 h per field. Measurements on 20 plants per key weed species (3–5 key weed species per field) are recommended to evaluate efficacy.

Other possible uses of the meters include the evaluation of herbicide injury to crop plants, herbicide sensitivity screening and classification, monitoring and preventing herbicide resistance development, predicting residual effects of soil herbicides and optimising herbicide dose. In the Netherlands, there is some experience with use of mobile photosynthesis meters in practice, in combination with the minimum lethal herbicide dose (MLHD) DSS (Kempenaar *et al.*, 2002; Kempenaar & van den Boogaard (2004)). MLHD is used to adjust herbicide dose to weed, crop and environmental conditions. At present, approximately 200 farmers and farm advisors in the Netherlands use leaf photosynthesis meters to evaluate and improve their decisions on herbicide dose with arable crops.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Annex S1** Additional information on leaf photosynthesis meters.

**Annex S2** Whole plant measurements with MIPS leaf photosynthesis imaging instrument.

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