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ORIGINAL ARTICLE

Evaluation of selected parameters of photosynthesis as herbicide stress indicators on the example of glyphosate

Karol Wysocki[1]*, Tadeusz Banaszkiewicz[2], Jacek Olszewski[3]

[1] Department of Horticulture, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

[2] Department of Entomology, Phytopathology and Molecular Diagnostics, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

[3] Didactic-Experimental Center, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

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*Corresponding address:
karol.wysocki@uwm.edu.pl

Abstract

This study was aimed at evaluating the effect of sublethal doses of glyphosate on physiological parameters of a common ornamental plant Mexican marigold (*Tagetes erecta*). The herbicide was applied in the following doses: 720 g · ha^{-1} (standard field dose), 144 g · ha^{-1}, 28.8 g · ha^{-1}, and 14.4 g · ha^{-1}, in the form of a spraying treatment of plants in a specialist spraying chamber. The net assimilation rate and leaf greenness index were then determined. Herbicide application in the sublethal doses, i.e. below 720 g · ha^{-1}, caused disorders in both analyzed physiological parameters of plants. The glyphosate dose of 144 g · ha^{-1} elicited transient disorders in the leaf greenness index. In turn, the use of the lower doses (28.8 g · ha^{-1} and 14.4 g · ha^{-1}) caused a short-term increase in the net photosynthesis rate in the plants which was accompanied by a decreased value of the leaf greenness index. Study results demonstrated the effect of sublethal doses of glyphosate as a stress factor in parameters associated with the process of photosynthesis in plants.

Keywords: glyphosate, leaf greenness index, N-phosphonomethyl glycine, net photosynthesis rate, sublethal dose

Introduction

Glyphosate (N-phosphonomethyl glycine) is a commonly known, non-selective post-emergence herbicide with a longstanding history of use (Duke and Powles 2008). It is absorbed by the foliar parts of plants and migrates through the phloem to roots (Klevorn and Wyse 1984). The mechanism of its action involves inhibiting the activity of the shikimic acid pathway (EPSPS synthase), which thus interferes with the biosynthesis of phenylalanine, tyrosine, and tryptophan (Rubin *et al.* 1984). This results in the inhibition of protein biosynthesis and some secondary metabolites (Franz *et al.* 1997). Available literature also provides information regarding the intermediate effect of glyphosate on the photosynthesis process. This compound was reported to reduce the contents of chlorophyll a and b in plants, to impede electron transfer in PS II photosynthesis, and to decrease the intensity of photosynthesis in such plants as: cogon grass (*Imperata cylindrical* L.) (Huang *et al.* 2012), giant cane (*Arundo donax* L.) (Santin-Montanya *et al.* 2013), or in glyphosate-resistant soya (*Glycine* Willd.) (Krenchinski *et al.* 2017). Some studies have also demonstrated changes in chlorophyll fluorescence (Zhang *et al.* 2015), cell membrane permeability and chloroplast pigment content in plants treated with an herbicide dose of 150 g · ha^{-1} (Silva *et al.* 2014). Glyphosate treatment was also found to cause disorders in gas exchange parameters and the activity of antioxidative enzymes in peanut leaves (*Arachis hypogaea* L.) (Radwan and Feyez 2016). Investigations conducted by Mateos-Naranjo and Perez-Martin (2013) demonstrated a suppressed photosynthetic activity of sea clubrush (*Bolboschoenus maritimus)* in response to substrat contamination with N-phosphonomethyl glycine (5–30 mg · l^{-1}).

The minimum effective dose of glyphosate is usually reported to be 540 g · ha^{-1}. Studies conducted so far have demonstrated that its sub-lethal doses may lead to delayed blooming and to the inhibition of pollen fertility in sensitive plants (Londo *et al.* 2014). Some reports show that low doses of glyphosate may stimulate the physiological processes of plants. Glyphosate treatment in the concentrations of 50–200 µg · ml^{-1} resulted in the stimulation of photosynthetic electron transport in blue algae (*Anabaena doliolum*) (Shiha and Singh 2004). It was also reflected in an increased dry matter content of the plants of scentless mayweed (*Tripleurospermum inodorum*) and chickweed (*Stellaria media*) (Cedergreen *et al.* 2007). Experiments carried out by Pokhrel and Karsai (2015) demonstrated a positive effect of long-term exposure to a sublethal concentration of a Roundup preparation (1–5%) on biomass growth in air plants (*Bryophyllum pinnatum*).

The objective of this study was to evaluate the effect of reduced doses of glyphosate (applied in the form of a Glyfos 360 SL preparation) on selected physiological parameters of Mexican marigold (*Tagetes erecta*).

Materials and Methods

Pot experiments with Mexican marigold (*T. erecta*) were carried out in a greenhouse of the Department of Horticulture at the University of Warmia and Mazury in Olsztyn. During the 3rd week of April flowers were planted individually in a universal substrate in 346 cm^2 pots (21 cm in diameter and 3 liters in volume). Two replicate experiments were planted separately with groups randomized and rotated in position on different greenhouse cultivation benches. The experiments were conducted in six replications at a controlled temperature (24°C/12 h + 16°C/12 h) and humidity – 50%. Plants were fertilized with a balanced nutrient medium having electrical conductivity (EC) parameters of 1.2 mS · cm^{-1} and pH of 6.5. The herbicide used in the study was a Glyfos 360 SL preparation (produced by Cheminova A/S Denmark), with glyphosate (N-phosphonomethyl glycine) as the active substance. The following experimental variants were tested:
C – control (without spraying),
D1 – glyphosate in a dose of 14.4 g · ha^{-1},
D2 – glyphosate in a dose of 28.8 g · ha^{-1},
D3 – glyphosate in a dose of 144 g · ha^{-1},
D4 – glyphosate in a dose of 720 g · ha^{-1} (corresponding to a field dose of 720 g · ha^{-1}).

The preparation was applied in a special spraying chamber using an XR TeeJet 11003 sprayer, at a working pressure of 4 bars, field speed of a spraying cart 4 km · h^{-1}, and spray rate of 400 l · ha^{-1}. The plants were sprayed during flower bud development, in the 10th week after planting in containers. The plants were analyzed for:
– leaf greenness index (SPAD) – using a Chlorophyll Meter SPAD-502 (Soil-Plant Analysis Development) (Minolta Co., Osaka, Japan). This device measures the differences between light absorption at 650 and 940 nm, and the quotient of these values is represented by the leaf greenness index or the chlorophyll content (Blackmer and Schepers 1995). The value of the SPAD readings is highly, positively correlated with the presence of chlorophyll, which can be used to determine the chlorophyll level (Chapman and Baretto 1997). Ten measurements were made of fully developed leaves of each plant: 3, 6, 10, 13, and 16 days after herbicide application, according to the producer's guidelines. The level of chlorophyll was expressed in dimensionless SPAD units;
– net photosynthesis rate – measured using a portable device for measurements of gas exchange parameters Li-Cor 6400 (DMP AG SA LTD). Measurements were conducted at a constant CO_2 concentration of 400 ppm, air temperature 25–28°C, and light irradiance of 1000 µmol · m^{-2} · s^{-1}. The source of photons was the lamp "LED Light Source" emitting light with the main peak spectrum concentrated in the band 670 nm and the second which was smaller – 465 nm. Readings were performed on fully developed leaves, in six replications of each object of the study. Values of the parameter were read out 3, 6, and 10 days after herbicide treatment, and the study results were presented as average values.

Study results concerning SPAD and net photosynthesis rate were subjected to a one-way analysis of variance ANOVA, using Statistica 12.0 software. The significance of differences was evaluated with the Tukey`s test, at a significance level of $\alpha = 0.01$.

Results

Analysis of experimental results indicated the utility of Mexican marigold (*T. erecta*) for the assessment of glyphosate impact on plants. The leaf greenness index on the day of herbicide application was at a similar level in all the examined objects (Fig. 1). During the studies a drop in the net photosynthesis rate values was observed in the control objects (Table 1) despite the relatively constant level of the leaf greenness index (from 49 to 52.6 SPAD). The application of glyphosate in all analyzed doses (720, 144, 28.8, and

Table 1. Results of net photosynthesis rate in Mexican marigold (*Tagetes erecta*) leaves treated with glyphosate

Dose of glyphosate [g · ha⁻¹]	Photosynthesis intensity [μmol CO_2 $m^{-2} \cdot s^{-1}$]								
	3 DAA*			6 DAA			10 DAA		
	1st exp.	2nd exp.	average	1st exp.	2nd exp.	average	1st exp.	2nd exp.	average
Control	7.47 bc	7.58 bc	7.52 BC	6.48 bc	6.76 bc	6.62 BC	5.69 c	5.72 b	5.70 B
D1= 14.4	8.15 c	8.22 cd	8.19 CD	7.07 c	7.25 cd	7.16 C	5.27 bc	5.38 b	5.33 B
D2 = 28.8	8.23 c	8.45 d	8.34 D	7.37 c	7.66 d	7.51 C	5.43 c	5.36 b	5.40 B
D3 = 144	6.85 ab	7.01 b	6.93 B	5.91 ab	6.16 b	6.03 AB	4.47 ab	4.58 a	4.53 A
D4 = 720	5.83 a	6.04 a	5.93 A	5.07 a	5.22 a	5.14 A	4.36 a	4.15 a	4.25 A

The same letters in a column mark averages which do not differ at α = 0.01 significance level
*DAA – days after application

14.4 g · ha⁻¹) caused a decrease in the leaf greenness index of marigold as early as 3 days after herbicide treatment (Fig. 2). This tendency was also observed in the two successive measurements (Figs. 3–4). The plants exposed to a glyphosate D4 dose (720 g · ha⁻¹) died 13 days after the treatment. During the same period, the plants from variants D1 and D2 were characterized by an increased chlorophyll level in leaves which was similar to that of the control plants (Fig. 5). This tendency was confirmed by the results of leaf greenness index measurements made 16 days after herbicide application (Fig. 6). At the same time, there was also an increase of the leaf greenness index in the D3 plants to the initial level. A different type of change was observed in the net photosynthesis rate (Table 1). The plants exposed to glyphosate doses of variants D1 and D2 responded to the stress factor with an 8.9 and 11.3% increase in the net photosynthesis rate, respectively, in the first term of evaluation as well as by 8.1 and 13.4% 6 days after herbicide treatment. The highest increase in the tested

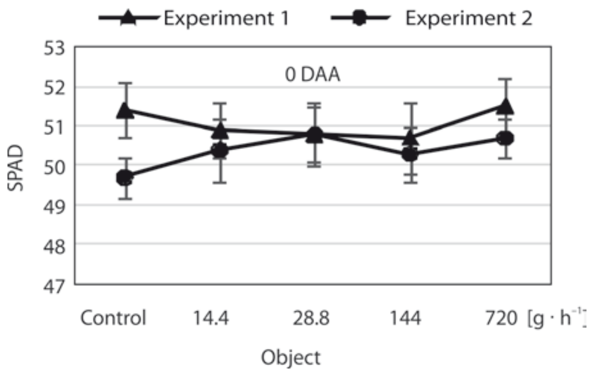

Fig. 1. Leaf greenness index (SPAD) of Mexican marigold (*Tagetes erecta*) treated with glyphosate at the application day (0 DAA)

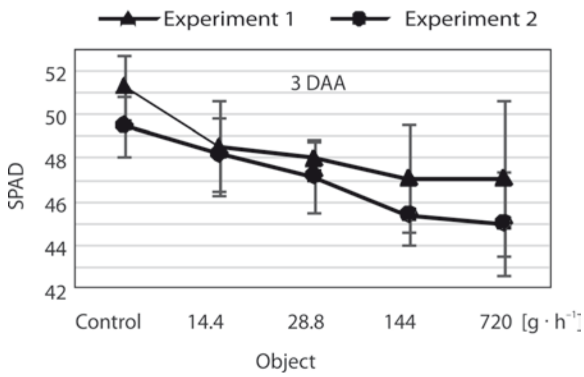

Fig. 2. Leaf greenness index (SPAD) of Mexican marigold (*Tagetes erecta*) treated with glyphosate at 3 days after application (3 DAA)

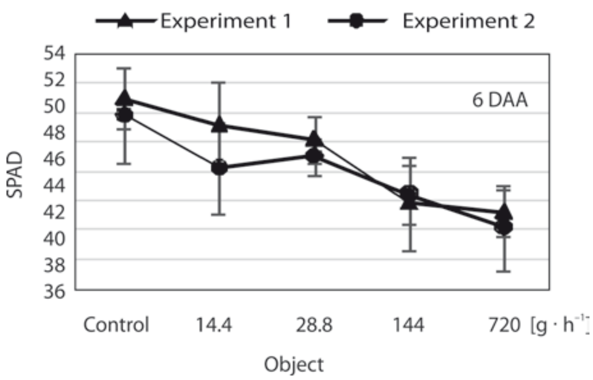

Fig. 3. Leaf greenness index (SPAD) of Mexican marigold (*Tagetes erecta*) treated with glyphosate at 6 days after application (6 DAA)

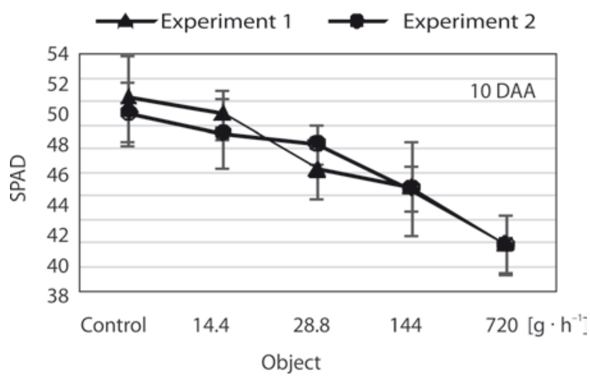

Fig. 4. Leaf greenness index (SPAD) of Mexican marigold (*Tagetes erecta*) treated with glyphosate at 10 days after application (10 DAA)

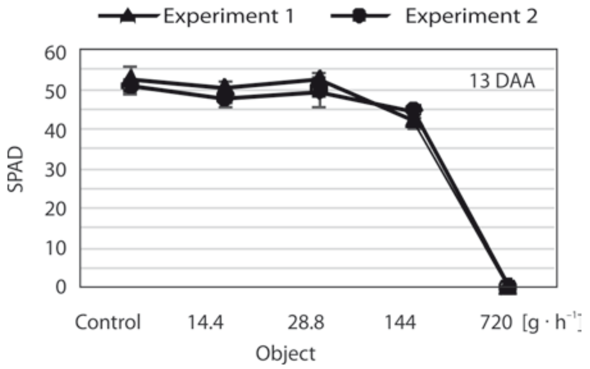

Fig. 5. Leaf greenness index (SPAD) of Mexican marigold (*Tagetes erecta*) treated with glyphosate at 13 days after application (13 DAA)

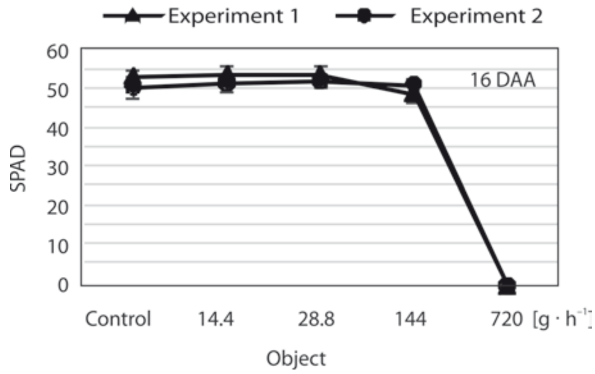

Fig. 6. Leaf greenness index (SPAD) of Mexican marigold (*Tagetes erecta*) treated with glyphosate at 16 days after application (16 DAA)

parameter was noted in the plants from the D2 variant in experiment 2. No differences were found in the values of this parameter in the third term of observations. In variants D3 and D4, values of this parameter were observed to decrease on all days of measurements but more visible changes were observed in the D4 experiment, where the dose of 720 · ha^{-1} was used.

Discussion

Measurements of such parameters as net photosynthesis rate and leaf greenness index brought robust results in the evaluation of the glyphosate effect on the analyzed plant. The application of the lower than recommended dose of the herbicide (in this study: glyphosate in a dose of 144 g · ha^{-1}) initially caused a decrease in the leaf greenness index and in the net photosynthesis rate, however these changes were temporary. It was observed that 16 days after herbicide treatment the leaf greenness index in these plants returned to the level noted in the control variant. The initial increase in the net photosynthesis rate and gas exchange was reported by De Carvalho *et al.* (2012) in coffee plants treated with glyphosate doses ranging from 180 to 360 g · ha^{-1}, but these changes subsided 60 days after herbicide application. This indicates that the use of sublethal doses of the discussed compound may lead to regressing physiological disorders in plants as a response of the organism to the stress factor. The phenomenon of hormesis, i.e. a stimulating effect of very low doses of a toxic preparation on a plant, was achieved with glyphosate treatment doses of 28.8 g · ha^{-1} and 14.4 g · ha^{-1}. It elicited a significant increase in the net photosynthesis rate in the first two terms of assessment. It confirms findings reported by other authors who achieved positive effects of low doses of glyphosate in their experiments (Belz and Leberle 2012). For instance, an increased chlorophyll content of goosefoot (*Chenopodium album*) leaves was demonstrated by Ketel (1996) after glyphosate application in doses of 180 and 90 g · ha^{-1}. In turn, Wong (2000) showed that glyphosate treatment in a dose of 0.02 mg · ha^{-1} had a stimulating effect on organism growth, net photosynthesis rate, and chlorophyll content in *Scenedesmus quadricauda* algae. Finally, Cedergreen and Olesen (2010) noted an increase in gas exchange and photosynthesis in barley plants exposed to glyphosate doses ranging from 11 to 45 g · ha^{-1}.

Conclusions

1. Mexican marigold (*T. erecta*) displayed high sensitivity to glyphosate applied in doses ranging from 14.4 to 720 g · ha^{-1}.
2. The first symptoms of herbicide effect were observed 3 days after its application at a rate of 2.7% of its minimum effective dose.
3. Herbicide stress was manifested by a short-term stimulation of the net photosynthesis rate accompanied by a decreased value of the leaf greenness index.

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