Acta Agrobotanica

DOI: 10.5586/aa.1730

Publication history
Received: 2017-07-19
Accepted: 2018-01-01
Published: 2018-03-13

Handling editor
Marzena Parzymies, Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin, Poland

Authors' contributions
MH, HD: concept and experimental protocol for the study; DK, MS: experiments; MS: data analysis; HD, JK, MH: manuscript preparation

Funding
This study was supported by the Polish Ministry of Science and Higher Education for the statutory activities (No. 13/91/S) of the Faculty of Natural Sciences, Siedlce University of Natural Sciences and Humanities.

Competing interests
No competing interests have been declared.

Citation
Dębski H, Szwed M, Koczkodaj D, Klocek J, Horbowicz M. Comparison of the response of seedlings of common buckwheat (*Fagopyrum esculentum* Moench) to glyphosate applied to the shoot or to the root zone. Acta Agrobot. 2018;71(1):1730. https://doi.org/10.5586/aa.1730

ORIGINAL RESEARCH PAPER

Comparison of the response of seedlings of common buckwheat (*Fagopyrum esculentum* Moench) to glyphosate applied to the shoot or to the root zone

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Abstract

We examined the response of common buckwheat (*Fagopyrum esculentum* Moench) seedlings, as a nontarget plant, to various doses of glyphosate applied to the root zone or to the shoots. Glyphosate was used at 0.1, 0.5, and 1.0 mM concentrations. The study was conducted on seedlings grown in hydroponic cultures under controlled growth conditions. Primary root and shoot growth, anthocyanin and photosynthetic pigment contents were measured to assess the effects of exposure to glyphosate. Glyphosate applied to shoots had a considerably higher impact on the growth of primary roots and shoots of seedlings. Low glyphosate concentrations produced an increase in anthocyanin content of hypocotyls, regardless of the mode of its application. Increasing the concentration of glyphosate applied to the root zone resulted in a gradual increase in anthocyanin content in cotyledons. Our overall results show that in hydroponically grown common buckwheat seedlings, glyphosate is less phytotoxic when applied to the root zone than when applied to the shoot. Low doses of glyphosate applied to the root zone stimulate root and shoot growth and increase the anthocyanin levels in cotyledons. The phytotoxicity of glyphosate was decreased in the absence of mineral nutrients in the root zone of buckwheat seedlings.

Keywords

herbicide; mode of application; common buckwheat; growth; anthocyanins; chlorophylls; carotenoids

Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] is an active ingredient in the herbicides which are the most widely used in the world. They are nonselective and postemergence, and so have broad-spectrum application [1]. Glyphosate-based formulations have not only been used in agriculture, but also in forestry and recreational areas [2]. They destroy nearly all herbaceous plants and are now the world's best-selling herbicides [1]. The worldwide increase in genetically-modified and glyphosate-resistant plants has been accompanied by an increase in glyphosate usage [3]; annual global production exceeds 1 million tones. Its ongoing use poses a significant risk to the environment and subsequent planting [4].

The effect of glyphosate on plants has been known since the early 1970s [5]. The enzymatic activity of the shikimate pathway in a glyphosate-exposed plant is inhibited, so blocking the synthesis of aromatic amino acids and a range of essential secondary metabolites such as flavonoids [5,6]. In addition, glyphosate inhibits chlorophyll biosynthesis as it blocks δ-aminolevulinic acid synthesis [7,8]. Together, these effects lead to rapid growth inhibition and ultimately plant death.

In both soils and plants, glyphosate degrades to aminomethylphosphonic acid [9,10]. It is commonly believed that glyphosate degradation is rapid and its accumulation in nature is low [1]. However, such conclusions are generally based on either laboratory bioassays or short-term field experiments [3]. Studies on herbicide residues in boreal environments have demonstrated that residues of glyphosate can be found in soils even years after application [2]. Glyphosate is mainly applied to plant leaves through spraying, and so enters the plant via diffusion [1]. In the field, following precipitation, glyphosate infiltrates into the soil and thus the roots of nontarget plants may be exposed to its residues. It may be taken up by plant roots and transported within the xylem via the apoplastic pathway [9]. Glyphosate absorption through roots has been demonstrated in several crop species [11,12]. Although plants can take up glyphosate through their roots, only small amounts of this herbicide are available via this pathway [13].

Glyphosate residues may contribute to severe impairment in the nutrition of nontarget plants due to formation of poorly soluble glyphosate–metal complexes [6,14,15]. The negative effects of foliar exposure on nontarget plants are well known [6,15,16], but there have been only a few studies in which plants were exposed to glyphosate present in the root zone [17,18]. A different response following glyphosate application to roots or shoots has been reported in maize seedlings [11]. An inhibitory effect of glyphosate on the biosynthesis of anthocyanins and other flavonoids in buckwheat seedlings has been known for many years [19]. Foliar-applied glyphosate inhibited almost the entire light-induced accumulation of phenylpropanoid substances in an etiolated buckwheat hypocotyl [19]. However, to date, no studies have been published on the effects of different modes of glyphosate application on the physiological and metabolic impacts in seedlings of this crop plant.

The aim of this study was to examine the response of common buckwheat seedlings, as nontarget plants, exposed to glyphosate at low and high doses applied to the root zone or sprayed on to shoots. In order to evaluate the effect of nutrients on glyphosate efficacy, seedling growth was compared in both a nutrient medium and in water without minerals. This response was evaluated by measuring the growth of the primary root and shoot as well as by the determination of anthocyanins and photosynthetic pigments in seedling cotyledons and hypocotyls.

Material and methods

Plant material

Seedlings of common buckwheat (*Fagopyrum esculentum* Moench 'Hruszowska') were used in this study. Seeds were germinated by placing them between two layers of wet filter paper which were then rolled up and inserted into a beaker containing tap water. The units were kept in darkness at 24 ±1°C for a period of 4 days. Following germination, uniform seedlings were selected and transferred to a controlled-environment room with a 16-h photoperiod and a day/night temperature regime of 24 ±2°C / 16 ±2°C. High-pressure sodium lamps (Plantaster 400W E40; Osram, Germany) provided a photon flux density of 100 ±20 μmol m^{-2} s^{-1}. After 1 day in these conditions, the seedlings were used in three experiments. In the first and second experiments, glyphosate was applied to the root zone of seedlings grown in water or nutrient medium. The glyphosate addition was chosen to produce 0.1, 0.5, or 1.0 mM herbicide concentrations in the root zone. In the third experiment, the shoots (hypocotyls and cotyledons) of seedlings grown in the nutrient medium were immersed in 0.1, 0.5, or 1.0 mM herbicide solution for 10 seconds.

Before using glyphosate and after 3- and 7-day periods following herbicide application, the lengths of upper parts (shoots) and primary roots of the seedlings were measured. The difference between the length of roots and shoots before and after 3 and 7 days following herbicide application was considered to be seedling elongation. For these measurements, 25–30 treated and controls seedlings were taken. After 7 days of the experiment, the lengths of shoots and primary roots were measured, and anthocyanins and photosynthetic pigments determined.

Determination of anthocyanins and photosynthetic pigments

Anthocyanins were extracted and measured as described by Mancinelli [20]. Absorbance of the extract was measured at 530 and 657 nm. The formula $A_{530} - 0.25A_{657}$ was used to compensate for the absorption of chlorophyll degradation products. Anthocyanin content was calculated using the molecular extinction coefficient of cyanidin-3-glucoside, $\varepsilon = 26{,}900$ [21]. The content of chlorophylls and total carotenoids in cotyledons was quantified spectrophotometrically using the method and extinction coefficients proposed by Lichtenthaler and Welburn [22]. In the case of anthocyanins, three independent replicates were analyzed for hypocotyls and cotyledons separately. The photosynthetic pigments were analyzed in three replicates of seedling cotyledons which had been treated with glyphosate by immersion of their shoots.

Statistical analysis

Two-way analysis of variance (ANOVA) and Tukey's post hoc test were used to check the significance of differences in the growth or anthocyanins content of seedlings exposed to glyphosate doses for each mode of application. For photosynthetic pigments analyses, one-way ANOVA was performed. Treatment effects were regarded as statistically significant at $p \leq 0.05$. Calculations were performed using the Statistica 12 software package (StatSoft, Poland).

Results

Glyphosate applied to the root zone after 3 days had only a weak impact on shoot length of seedlings grown in either water or nutrient solution (Tab. 1). Only the higher concentrations of the herbicide (0.5 and 1.0 mM) inhibited the growth of shoots. However, after a longer (7 day) exposure to the herbicide, elongation of shoots was significantly decreased in comparison to control seedlings. Shoot application of glyphosate had a considerably higher impact on shoot elongation than when applied in the root zone. The 3-day exposure of shoots to 1 mM glyphosate resulted in a 2.5-fold reduction in shoot elongation, and after 7 days of treatment it was almost fivefold.

Three-day exposure to glyphosate applied to the root zone had a major impact on the root elongation of seedlings independently of the mode the herbicide application (Tab. 2).

However, glyphosate applied to the shoots inhibited root growth significantly more than when applied to the root zone. After 3 days of exposure to the lowest glyphosate concentration, root elongation was fourfold lower compared to the control; after 7 days, the primary root elongation was 7 times lower than in the control seedlings. Higher concentrations of glyphosate applied to shoots almost inhibited elongation of the primary roots.

Comparison of the results for 7-day exposure to glyphosate revealed marked differences between the lengths of primary roots and shoots (Tab. 3). When glyphosate was applied to the root zone of seedlings grown in water without minerals, the inhibition of root growth was small and statistically insignificant. When the herbicide was applied to the root zone of seedlings grown in the presence of mineral nutrients, inhibition of primary root growth was greater, especially at the highest concentration (1 mM). The application of glyphosate to shoots resulted in a significant inhibition of the growth of shoots and primary roots even at the lowest concentration of the herbicide (0.1 mM).

The lowest glyphosate concentration (0.1 mM) increased the content of anthocyanins in hypocotyls (Fig. 1A–C). The intermediate dose (0.5 mM) applied to the root zone caused nonsignificant changes in the content of these pigments (Fig. 1A,B). The highest (1.0 mM) concentration of glyphosate, regardless of the application method, produced a marked decline in anthocyanin content. Glyphosate applied to the shoots had the greatest impact on reduction of anthocyanin content in the hypocotyl. At 0.5 and 1.0 mM concentrations of the herbicide, the anthocyanin content was approximately 3–4 times

Tab. 1 Effect of the method of glyphosate application on shoot elongation (mm; mean ±standard deviation) of common buckwheat seedlings after 3 and 7 days of treatment.

Treatment	Glyphosate applied to roots grown in water	Glyphosate applied to roots grown in nutrient solution	Glyphosate applied on shoot of seedlings grown in nutrient solution
Three days after treatment			
Control	32.4 ±7.4 [abc]	38.8 ±12.3 [a]	36.2 ±8.7 [abc]
Glyphosate 0.1 mM	37.2 ±13.0 [ab] (114)	39.1 ±10.1 [a] (101)	30.3 ±9.9 [bc] (84)
Glyphosate 0.5 mM	30.3 ±11.3 [bc] (94)	28.9 ±8.5 [bc] (74)	16.8 ±6.4 [d] (46)
Glyphosate 1.0 mM	29.8 ±11.2 [bc] (92)	28.3 ±8.6 [c] (73)	13.8 ±3.9 [d] (38)
Seven days after treatment			
Control	48.6 ±12.9 [cde]	63.0 ±24.0 [b]	76.4 ±17.4 [a]
Glyphosate 0.1 mM	53.3 ±16.0 [bc] (110)	52.0 ±14.3 [bcd] (82)	51.2 ±16.4 [bcd] (67)
Glyphosate 0.5 mM	39.8 ±11.8 [def] (82)	38.2 ±10.8 [ef] (61)	21.3 ±8.7 [g] (28)
Glyphosate 1.0 mM	36.3 ±12.4 [f] (75)	33.9 ±10.13 [f] (54)	16.5 ±4.7 [g] (22)

Means within a column followed by the same letter are not significantly different at the $p \leq 0.05$ level as determined by two-way analysis of variance with the Tukey's post hoc test after 3 and 7 days, separately. Percentages relative to the control are shown in parentheses.

Tab. 2 Effect of the method of glyphosate application on primary root elongation (mm; mean ±standard deviation) of common buckwheat seedlings after 3 and 7 days of treatment.

Object	Glyphosate applied to roots grown in water	Glyphosate applied to roots grown in nutrient solution	Glyphosate applied on shoot of seedlings grown in nutrient solution
Three days after treatment			
Control	23.0 ±17.3 [bc]	23.8 ±18.2 [ab]	32.3 ±19.4 [a]
Glyphosate 0.1 mM	20.5 ±7.8 [bcd] (89)	13.2 ±6.2 [de] (55)	8.1 [ef] (25)
Glyphosate 0.5 mM	14.7 ±5.7 [cde] (64)	9.1 ±4.4 [ef] (38)	4.0 [f] (12)
Glyphosate 1.0 mM	10.9 ±5.0 [ef] (47)	6.6 ±3.9 [ef] (28)	3.3 [f] (10)
Seven days after treatment			
Control	29.8 ±19.9 [b]	33.0 ±21.5 [b]	63.7 ±31.6 [a]
Glyphosate 0.1 mM	24.4 ±9.1 [c] (85)	16.0 ±7.3 [cde] (48)	9.0 ±4.9 [de] (21)
Glyphosate 0.5 mM	17.4 ±5.7 [cd] (58)	10.4 ±4.2 [de] (32)	4.5 ±1.9 [e] (10)
Glyphosate 1.0 mM	13.9 ±5.9 [cde] (47)	9.7 ±4.8 [de] (29)	4.0 ±1.8 [e] (9)

Means within the table followed by the same letter are not significantly different at the $p \leq 0.05$ level as determined by two-way analysis of variance with the Tukey's post hoc test after 3 and 7 days, separately. In the parentheses is shown the percentage relative to the control.

Tab. 3 Effect of the method of glyphosate application on primary roots and shoots length (mm; mean ±standard deviation) of common buckwheat seedlings (7 days of treatment).

Object	Glyphosate applied to roots grown in water	Glyphosate applied to roots grown in nutrient solution	Glyphosate applied on shoot of seedlings grown in nutrient solution
Length of primary roots			
Control	74.0 ±32.0 [bcd]	93.0 ±37.4 [b]	118.3 ±39.4 [a]
Glyphosate 0.1 mM	68.7 ±15.5 [cd] (93)	67.4 ±18.3 [cd] (72)	86.0 ±21.9[b c] (73)
Glyphosate 0.5 mM	59.5 ±16.1 [d] (80)	75.6 ±16.8 [bcd] (81)	65.4 ±19.0 [cd] (55)
Glyphosate 1.0 mM	61.7 ±19.8 [d] (83)	71.2 ±17.4 [cd] (77)	62.8 ±23.5 [d] (53)
Length of shoots			
Control	85.5 ±12.9 [de]	129.2 ±27.0 [a]	118.3 ±21.1 [ab]
Glyphosate 0.1 mM	83.7 ±20.8 [def] (98)	111.6 ±22.7 [bc] (86)	95.6 ±18.5 [cd] (81)
Glyphosate 0.5 mM	72.0 ±13.8 [efg] (84)	100.5 ±20.4 [cd] (78)	68.6 ±17.8 [efg] (58)
Glyphosate 1.0 mM	67.3 ±18.9 [fg] (79)	91.0 ±24.5 [d] (70)	60.1 ±13.3 [g] (51)

Means within the table followed by the same letter are not significantly different at the $p \leq 0.05$ level as determined by two-way analysis of variance with the Tukey's post hoc test for length of primary roots and shoots, separately. In the parentheses is shown the percentage relative to the control.

lower relative to that of control seedlings (Fig. 1C). An increase in the concentration of glyphosate applied to the root zone led to a gradual increase in anthocyanin content in the cotyledons (Fig. 2A,B). This was particularly noticeable in seedlings grown in water (Fig. 2A). However, unlike the hypocotyl, even the lowest concentration of the herbicide (0.1 mM) applied to shoots contributed to an approximately 40% reduction in the level of anthocyanins compared to the cotyledons of control plants (Fig. 2C).

A low concentration of glyphosate applied to shoots increased chlorophylls *a* and *b* contents (Tab. 4). However, this concentration of glyphosate did not increase the content of total carotenoids. Higher doses of herbicide contributed to a significant decline in the levels of the chlorophylls but the reduction of the total carotenoids was not so great. An increased ratio of chlorophyll *a*:*b* indicates a more pronounced

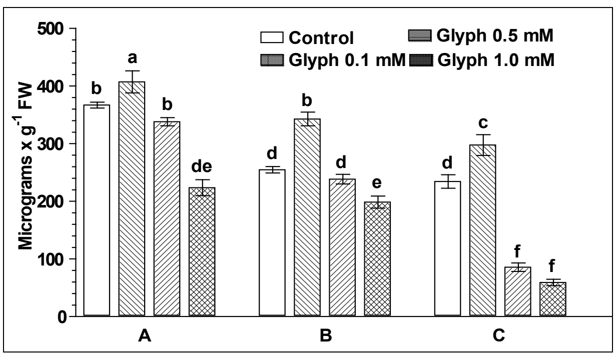

Fig. 1 Effect of the method of glyphosate (Glyph) on the content of anthocyanins in hypocotyl of buckwheat seedlings after 7-day treatment. (**A**) Glyphosate applied to roots grown in water; (**B**) glyphosate applied to roots grown in nutrient solution; (**C**) glyphosate applied to shoots of seedlings grown in nutrient solution. Means followed by the same letter are not significantly different at the $p \leq 0.05$ level as determined by two-way analysis of variance with the Tukey's post hoc test.

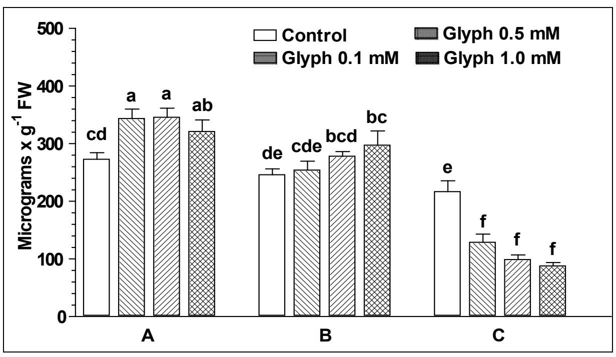

Fig. 2 Effect of the method of glyphosate (Glyph) on the content of anthocyanins in cotyledons of buckwheat seedlings after 7-day treatment. (**A**) Glyphosate applied to roots grown in water; (**B**) glyphosate applied to roots grown in nutrient solution; (**C**) glyphosate applied to shoots of seedlings grown in nutrient solution. Means followed by the same letter are not significantly different at the $p \leq 0.05$ level as determined by two-way analysis of variance with the Tukey's post hoc test.

Tab. 4 Effect of 7-day treatment with glyphosate applied to shoots on the content (mean ±standard deviation) of photosynthetic pigments in cotyledons of buckwheat seedlings.

	Content of pigments ($\mu g\ g^{-1}$ fresh weight)			Ratio	
Object	Chlorophyll *a*	Chlorophyll *b*	Total carotenoids	Chl *a* / Chl *b*	Chl *a* / total carotenoids
Control	1215.4 ±30.2 ^{b}	484.7 ±18.7 ^{a}	230.5 ±6.7 ^{a}	2.51	5.27
Glyphosate 0.1 mM	1342.4 ±28.5 ^{a} (110)	520.2 ±11.5 ^{a} (107)	218.3 ±1.6 ^{a} (95)	2.58	6.15
Glyphosate 0.5 mM	781.0 ±31.6 ^{c} (64)	289.8 ±16.0 ^{b} (60)	174.7 ±2.0 ^{b} (76)	2.69	4.47
Glyphosate 1.0 mM	561.0 ±26.4 ^{d} (46)	203.8 ±20.9 ^{c} (42)	160.2 ±7.0 ^{c} (70)	2.75	3.50

Means followed by the same letter are not significantly different at the $p \leq 0.05$ level as determined by one-way analysis of variance with the Tukey's post hoc test, calculated for each table column separately. In the parentheses is shown the percentage relative to the control.

effect of glyphosate on the biosynthesis of chlorophyll *b*. On the other hand, the lower chlorophyll *a* : total carotenoids ratio indicates that the carotenoid pigments are more resistant to the herbicide than are the chlorophylls.

Discussion

Plants grown in a hydroponic medium containing glyphosate can take up the herbicide through their roots [12,13]. In maize (*Zea mays* L.) seedlings, glyphosate taken up by the roots is primarily transported to the shoot apex [11]. However, only small amounts of the herbicide are available via this pathway [13]. It is likely that the roots of common buckwheat seedlings also take up only small amounts of glyphosate. However, it has been established that low doses of glyphosate can stimulate the growth of many plant species [6,13,18,23,24]. The results of the present work support findings of these earlier studies. The phenomenon of hormesis is common in many plant species [25].

Higher sensitivity to glyphosate of root growth compared to that of shoots has been established previously [12,26,27]. Piotrowicz-Cieślak et al. [18] studied the impact of glyphosate applied to soil on the germination and growth of seedlings of several species. They found that the root length can be an important indicator of the presence of glyphosate residues in soil. The results of present work confirmed these findings. However, it is more difficult to explain why seedlings grown in the nutrient medium grew slower in the presence of glyphosate than the seedlings grown without nutrients. Perhaps mineral uptake by the roots of the seedlings also facilitated glyphosate uptake. On the other hand, plants that grow in the nutrient deficient conditions are under stress. This stress may stimulate various defence mechanisms which can also counteract the activity of the herbicide. Furthermore, glyphosate is known to be a cation chelator forming complexes with certain nutrient elements, thus making them unavailable for plants. For example, cations of Cu and Zn in solution can be strongly complexed by glyphosate [14,17]. The presence of glyphosate in the root zone, therefore, may substantially change the availability of the nutrient elements, resulting in a more pronounced effect of the herbicide. All these hypotheses do require further detailed testing.

An inhibitory effect of glyphosate on the biosynthesis of anthocyanins in plants has been known for many years [1,4,5,19]. The herbicide inhibits the shikimate pathway, which blocks the synthesis of aromatic amino acids and indirectly inhibits biosynthesis of their secondary products which include anthocyanins [6]. Strong inhibition of the biosynthesis of anthocyanins in the hypocotyl of buckwheat seedlings was recognized as far back as the 1970's [5,19]. Authors of these studies found that glyphosate inhibited the light-induced accumulation of chlorogenic acid, procyanidin, rutin, and anthocyanins in etiolated buckwheat hypocotyls. However, the stimulatory effect of a low dose of glyphosate on the accumulation of anthocyanins, which was found during present studies, is a new observation. The increase in anthocyanins content was particularly visible in the cotyledons when seedlings were grown in the nutrient medium. An explanation of this phenomenon can be the previously mentioned finding that nutrient deficiency increases the content of anthocyanins in plants [28]. This deficit may be due to the presence of glyphosate [14,17]. The herbicide also reduces chlorophyll content directly due to the inhibition of chlorophyll biosynthesis [7,8,29] or indirectly by decreasing the magnesium content of photosynthetic tissues [15]. The results of our study confirm these observations. An increase in chlorophyll content under low glyphosate dosage probably relates to the phenomenon of hormesis.

Conclusions

The results of the study discussed here suggest that glyphosate application may result in severe impairment of the growth of common buckwheat seedlings here used as nontarget plants. However, plant response to glyphosate is dependent on the application method as well as the concentration used. Based on the results obtained in this study, it is clear that glyphosate is less phytotoxic when applied to the root zone than to the shoot of buckwheat seedlings. Glyphosate applied to the root zone at low doses increased the growth of both primary roots and shoots. The phytotoxicity of glyphosate was decreased in the absence of mineral nutrients in the root zone. Glyphosate applied to the root zone increased the level of anthocyanins in the cotyledons but shoot application decreased its content.

References

1. Duke SO, Powles SB. Glyphosate: a once-in-a-century herbicide. Pest Manag Sci. 2008;64:319–325. https://doi.org/10.1002/ps.1518
2. Laitinen P, Rämö S, Siimes K. Glyphosate translocation from plants to soil – does this constitute a significant proportion of residues in soil? Plant Soil. 2007;300:51–60. https://doi.org/10.1007/s11104-007-9387-1
3. Helander M, Saloniemi I, Saikkonen K. Glyphosate in northern ecosystems. Trends Plant Sci. 2012;17:569–574. https://doi.org/10.1016/j.tplants.2012.05.008
4. Székács A, Darvas B. Forty years with glyphosate. In: Hasaneen MNAEG, editor. Herbicides – properties, synthesis and control of weeds. Rijeka: InTech; 2012. p. 247–284. https://doi.org/10.5772/32491
5. Amrhein N, Deus B, Gehrke P, Steinrücken HC. The site of the inhibition of the shikimate pathway by glyphosate II. Interference of glyphosate with chorismate formation in vivo and in vitro. Plant Physiol. 1980;66:830–834. https://doi.org/10.1104/pp.66.5.830
6. Duke SO, Lydon J, Koskinen WC, Moorman TB, Chaney RL, Hammerschmidt R. Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. J Agric Food Chem. 2012;60:10375–10397. https://doi.org/10.1021/jf302436u
7. Zobiole LH, Kremer RJ, Oliveira RS, Constantin J. Glyphosate affects chlorophyll, nodulation and nutrient accumulation of "second generation" glyphosate-resistant soybean (*Glycine max* L.). Pestic Biochem Physiol. 2011;99:53–60. https://doi.org/10.1016/j.pestbp.2010.10.005
8. Huang J, Silva EN, Shen Z, Jiang B, Lu H. Effects of glyphosate on photosynthesis, chlorophyll fluorescence and physicochemical properties of cogongrass (*Imperata cylindrical* L.). Plant Omics. 2012;5:177–183.
9. Franz JE, Mao MK, Sikorski JA. Glyphosate: a unique global herbicide. Washington, DC: American Chemical Society; 1997. (ACS Monograph; vol 189).
10. Reddy KN, Rimando AM, Duke SO, Nandula VK. Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. J Agric Food Chem. 2008;56:2125–2130. https://doi.org/10.1021/jf072954f

11. Alister C, Kogan M, Pino I. Differential phytotoxicity of glyphosate in maize seedlings following applications to roots or shoot. Weed Res. 2005;45:27–32. https://doi.org/10.1111/j.1365-3180.2004.00424.x
12. Petersen IL, Hansen HC, Ravn HW, Sørensen JC, Sørensen H. Metabolic effects in rapeseed (*Brassica napus* L.) seedlings after root exposure to glyphosate. Pestic Biochem Physiol. 2007;89:220–229. https://doi.org/10.1016/j.pestbp.2007.06.009
13. Wagner R, Kogan M, Parada AM. Phytotoxic activity of root absorbed glyphosate in corn seedlings (*Zea mays* L.). Weed Biol Manag. 2003;3:228–232. https://doi.org/10.1046/j.1444-6162.2003.00110.x
14. Eker S, Ozturk L, Yazici A, Erenoglu B, Römheld V, Cakmak I. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. J Agric Food Chem. 2006;54:10019–10025. https://doi.org/10.1021/jf0625196
15. Cakmak I, Yazici A, Tutus Y, Ozturk L. Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. Eur J Agron. 2009;31:114–119. https://doi.org/10.1016/j.eja.2009.07.001
16. White AL, Boutin C. Herbicidal effects on nontarget vegetation: investigating the limitations of current pesticide registration guidelines. Environ Toxicol Chem. 2007;26:2634–2643. https://doi.org/10.1897/06-553.1
17. Vereecken H. Mobility and leaching of glyphosate: a review. Pest Manag Sci. 2005;61:1139–1151. https://doi.org/10.1002/ps.1122
18. Piotrowicz-Cieślak AI, Adomas B, Michalczyk DJ. Different glyphosate phytotoxicity of seeds and seedlings of selected plant species. Pol J Environ Stud. 2010;19:123–129.
19. Holländer H, Amrhein N. The site of the inhibition of the shikimate pathway by glyphosate I. Inhibition by glyphosate of phenylpropanoid synthesis in buckwheat (*Fagopyrum esculentum* Moench). Plant Physiol. 1980;66:823–829. https://doi.org/10.1104/pp.66.5.823
20. Mancinelli AL. Photoregulation of anthocyanin synthesis. VIII. Effects of light pretreatments. Plant Physiol. 1984;75:447–453. https://doi.org/10.1104/pp.75.2.447
21. Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Int. 2005;88(5):1269–1278.
22. Lichtenthaler HK, Wellburn AR. Determination of total carotenoids and chlorophylls *a* and *b* of leaf in different solvents. Biochem Soc Trans. 1985;11:591–592. https://doi.org/10.1042/bst0110591
23. Cedergreen N. Is the growth stimulation by low doses of glyphosate sustained over time? Environ Pollut. 2008;156:1099–1104. https://doi.org/10.1016/j.envpol.2008.04.016
24. Velini ED, Alves E, Godoy MC, Meschede DK, Souza RT, Duke SO. Glyphosate applied at low doses can stimulate plant growth. Pest Manag Sci. 2008;64:489–496. https://doi.org/10.1002/ps.1562
25. Belz RG, Duke SO. Herbicides and plant hormesis. Pest Manag Sci. 2014;70:698–707. https://doi.org/10.1002/ps.3726
26. Cornish PS. Glyphosate residues in a sandy soil affect tomato transplants. Aust J Exp Agric. 1992;32:395–399. https://doi.org/10.1071/EA9920395
27. Lejczak B, Boduszek B, Kafarski P, Forlani G, Wojtasek H, Wieczorek P. Mode of action of herbicidal derivatives of aminomethylenebisphosphonic acid. I. Physiologic activity and inhibition of anthocyanin biosynthesis. J Plant Growth Regul. 1996;15:109–113. https://doi.org/10.1007/BF00198924
28. Krause J, Reznik H. Investigations on flavonol accumulation in *Fagopyrum esculentum* Moench as influenced by P- and N-deficiency. Zeitschrift für Pflanzenphysiologie. 1976;70:392–400. https://doi.org/10.1016/S0044-328X(76)80158-4
29. Silva FB, Costa AC, Alves RRP, Megguer CA. Chlorophyll fluorescence as an indicator of cellular damage by glyphosate herbicide in *Raphanus sativus* L. plants. Am J Plant Sci. 2014;5:2509–2519. https://doi.org/10.4236/ajps.2014.516265

Porównanie odpowiedzi siewek gryki zwyczajnej (*Fagopyrum esculentum* Moench) na glifosat stosowany na pędy lub do strefy korzeniowej

Streszczenie

Wykonano badania reakcji siewek gryki zwyczajnej (*Fagopyrum esculentum* Moench), na glifosat, związek chemiczny z grupy fosfonianów. Preparat zastosowano w stężeniach 0,1, 0,5 i 1,0 mM na organy nadziemne lub do strefy korzeniowej. Badania przeprowadzono na roślinach uprawianych hydroponicznie w kontrolowanych warunkach światła i temperatury. Aby ocenić wpływ ekspozycji na glifosat wykonano pomiary wzrostu korzenia głównego i części nadziemnych oraz zawartości antocyjanów i barwników fotosyntetycznych. Glifosat stosowany na organy nadziemne miał znacznie większy wpływ hamujący na wzrost tych organów i korzenia głównego siewek gryki, niż użyty dokorzeniowo. Niskie stężenie glifosatu (0,1 mM), niezależnie od trybu jego użycia, powodowało zwiększanie zawartości antocyjanów w hipokotylu siewek gryki zwyczajnej. Podwyższanie stężenia glifosatu w strefie korzeniowej powodowało stopniowy wzrost zawartości antocyjanów w liścieniach siewek. Uzyskane wyniki ukazują, że w uprawie hydroponicznej siewek gryki zwyczajnej glifosat jest mniej fitotoksyczny po użyciu do strefy korzenia, niż po zastosowaniu na organy nadziemne. Niskie dawki glifosatu (0,1 mM) w strefie korzeniowej stymulowały wzrost korzenia głównego i części nadziemnych siewek oraz zwiększały poziom antocyjanów w liścieniach. Fitotoksyczność glifosatu ulegała obniżeniu przy braku składników mineralnych w pożywce.