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EFFECT OF ROOT-ZONE GLYPHOSATE EXPOSURE ON GROWTH AND ANTHOCYANINS CONTENT OF RADISH SEEDLINGS

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ABSTRACT

The response of radish seedlings (*Raphanus sativus* L. subvar. *radicula* Pers.), as non-target plant, to various doses of glyphosate applied to root zone was studied in the experiment. The glyphosate was used at concentrations 0.1, 0.5 and 2.0 mM, and the study was conducted on seedlings grown in hydroponic cultures in controlled light and temperature conditions. In the experiment, roots of seedlings were exposed to glyphosate for 4, 7 or 14 days. In order to evaluate the effect of glyphosate, length and biomass of the seedling organs, as well as contents of anthocyanins in hypocotyls and cotyledons have been measured. Glyphosate applied to root zone had considerably higher impact on the growth of the primary root than shoot of radish seedlings. Short-term exposure to glyphosate led to the stimulation of growth and biomass organs of the radish seedling, but such treatment had no effect on the contents of anthocyanins in the cotyledons and hypocotyl. However, after longer exposure to glyphosate a decrease of anthocyanins content in the hypocotyl and its increase in the cotyledons was noted.

Key words: herbicide, dose, uptake, hormesis, *Raphanus sativus*

INTRODUCTION

The glyphosate (N-(phosphonomethyl) glycine) is an active ingredient of foliar applied herbicides which have been used intensively worldwide for about 45 years, being the most commercialized type of herbicides today [Komives and Schroder 2016]. Within a glyphosate and glyphosate-based herbicides exposed plant, the enzymatic action of the shikimate pathway is inhibited, which blocks the synthesis of aromatic amino acids and a variety of essential secondary metabolites, such as flavonoids [Duke et al. 2012]. These herbicides also stimulate the synthesis of ethylene, and as a result the synthesis of phospholipid degradation enzymes, and decrease the stability of cellular membranes [Silva et al. 2014].

After being applied on aerial parts of plants, glyphosate is readily transported through the phloem following the route of photosynthesis products, and moves to the growing parts of the plants like roots, tubers, young leaves and meristems [Hetherington et al. 1999]. The process is essential for herbicide effectiveness and may affect its efficacy [Satchivi et al. 2000].

Most of previous research concerns the foliar application of glyphosate. In the experiment of Basantani et al. [2011], there was a general decline in fresh weight and root length of the two *Vigna radiata* varieties after glyphosate treatment. Preharvest application of glyphosate in pea reduced seed germination,

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seedling emergence and shoot fresh weight [Baig et al. 2003]. Glyphosate inhibited almost completely the light-induced accumulation of anthocyanins in etiolated buckwheat hypocotyls [Amrhein et al. 1980]. Also Hoagland [1980] reported a substantial reduction in anthocyanins content when glyphosate was applied to soybean seedlings.

The effects of glyphosate depend on the concentration used. Glyphosate at concentration as in commonly used Round-up herbicide leads to fast chlorosis, followed by necrosis of the meristematic tissue, overall plant wilting, and eventually plant death during several days after treatment, but the first symptoms were observed one day after treatment [Ellis and Griffin 2002, Banaszkiewicz and Wysocki 2012, Mateos-Naranjo and Perez-Martin 2013].

Due to the widespread use and high quantity of glyphosate applied each year, the potential toxicity of its residues rises and may affect the succeeding plants. Therefore, glyphosate residues in soil may be important from both agricultural and ecotoxicological viewpoints. Glyphosate can affect levels of indole-3--acetic acid, and thus inhibit germination and seedling growth [Clay and Griffin 2000]. According to Piotrowicz-Cieślak et al. [2010] the root length can be an important indicator of presence of glyphosate residues in soil. They studied the effect of glyphosate on germination and growth of seedlings of following species: *Lepidium sativum*, *Sinapis alba*, *Sorghum saccharatum*, *Brassica napus*, *Lupinus luteus* and *Avena sativa*. Also, in experiments by Wagner et al. [2003] a close correlation between the response of young maize seedlings and glyphosate absorbed from the soil was found. According to Sacała et al. [2011] a small amount of the glyphosate residues in root zone markedly inhibited growth of non-target cucumber seedlings and significantly changed their metabolism. Especially the herbicide considerably suppressed the growth of cucumber roots.

Glyphosate is introduced to the soil either through a straight application, or when leached into the rhizosphere from root exudates of a glyphosate-treated plant. The zwitterionic character of the glyphosate molecule is responsible for its tendency to sorb strongly to organic matrices or clay minerals of soil. Phosphates compete with glyphosate for binding sites of soil. Because phosphate is preferentially sorbed, it may remobilize previously-bound glyphosate [Borggaard and Gimsing 2008]. This causes that experiments related to the absorption, translocation and activity of glyphosate when applied to roots are difficult to perform with credibility [Alister et al. 2005, Bott et al. 2011]. Therefore, such experiments are often conducted in hydroponic cultivation when glyphosate is applied to roots with aqueous solution.

Studies with low levels of glyphosate have found growth stimulation of some plant species [Duke et al. 2006; Velini et al. 2008]. Such a growth stimulant effect (hormesis) is an important factor in herbicide use and allelopathy [Duke et al. 2006]. The uptake of glyphosate into plants grown in soil containing residues of the herbicide was very low [Giesy et al. 2000; Dill et al. 2010]. These data demonstrate that a hormetic properties of glyphosate can occur when it is taken in this way.

A convenient object for assessing the impact of glyphosate residues are plant seedlings [Wagner et al. 2003, Alister et al. 2005, Piotrowicz-Cieślak et al. 2010, Banaszkiewicz and Wysocki 2012]. The objective of this study was to examine a response of hydroponically grown radish seedlings, as non-target plant, to various doses and time exposure of glyphosate applied to root-zone. This response was evaluated by measuring the growth of the seedling organs, as well as by contents of anthocyanins pigments in hypocotyl and cotyledons.

MATERIAL AND METHODS

The experiments were conducted on radish seedlings (*Raphanus sativus* L. subvar. *radicula* Pers., cv. Krakowianka) grown in hydroponic cultures. Initially, the seeds were subjected to 24 hour imbibition in water. Germination process was carried out in darkness at 24 ±1°C during 3 days. Germination was carried out by placing radish seeds between two layers of wet filter paper (30 × 6 cm) which were then rolled up and inserted in a beaker containing tap water. Ten seeds were germinated in each roll (total ca. 300). Following germination, about 200 of uniform seedlings (4–5 cm length) were selected, placed in 20 rolls of fresh wet filter paper immersed to a depth

of 2 cm in 1/5-strength Hoagland nutrient solution, and incubated for 24 h in growth room conditions (16 h photoperiod and temperature 24 ±2°C / 16 ±2°C, day/night). Light (100 ±20 μmol $m^{-2} \cdot s^{-1}$) was provided by high-pressure sodium lamps (Plantaster 400W E40, Osram, Germany). After one-day incubation in these conditions, the seedlings were used in the experiment. In the experiment the effect of 0.1; 0.2 or 2.0 mM concentration of glyphosate added to nutrient solution was evaluated. Control seedlings were grown in beakers containing only the Hoagland nutrient solution.

After 4, 7 and 14 days of experiment related to glyphosate exposure there were investigated growth parameters (length of roots and shoots, fresh weight of roots, hypocotyls and cotyledons) and content of anthocyanins in hypocotyls and cotyledons of the radish seedlings. Fifteen to twenty seedlings were taken for measurements of length and fresh mass of roots, cotyledons and hypocotyls. Each of these seedlings accounted for one replicate.

Total anthocyanins content was analyzed according to a method described by Mancinelli [1984]. Briefly, anthocyanins were extracted with 1% HCl-MeOH. In the extracts absorbance at 530 nm and 657 nm was measured. The formula $A_{530} - 0.25A_{657}$ was used to compensate for the absorption of chlorophyll degradation products. Anthocyanin content was expressed as μg of cyanidin 3-glucoside in 1 g of fresh matter, using 29,600 as molecular extinction coefficient. Six to eight replicates were analyzed (each contained tissues from two – three seedlings), for hypocotyls and cotyledons separately. Results of analyses were subjected to statistical evaluation according to Newman-Keuls test, $P \leq 0.05$.

RESULTS

The seedlings of radish survived under all examined glyphosate doses, but herbicide applied to root-zone markedly inhibited growth of both seedling organs after 7 and 14-day of exposure (tab. 1). The growth of radish shoot during these experiments was small, whereas root growth was very large (tab. 1). However, after 4-day exposure, the low doses of glyphosate (0.1 mM and 0.2 mM) significantly stimulated the shoot growth, which was higher, relative to the control, by 14% and 20%, respectively. But at the same time there was no effect of these doses of glyphosate on the growth of roots and whole seedlings. Compared to the control, the used herbicide concentration had no significant effect on shoot growth of seedling. After long exposure (14 days) the effect of glyphosate on the growth of roots and whole seedlings was very large, since the root length reached 16 to 24% length of the control seedlings.

Similarly to the length of shoot after 4-day exposure, a low glyphosate doses (0.1 mM and 0.2 mM) induced biomass accumulation in the organs of radish seedling, although generally it was not significant (tab. 2). The exception were hypocotyls treated with 0.1 mM of glyphosate, where there was a significant 28% increase in biomass accumulation in relation to control plants. Prolonged exposure (14 days) to herbicide inhibited mass increase in all organs of radish seedlings. Also in this case, the evaluated herbicide had largest impact on the fresh mass of roots. Root biomass was 39% of control when 2 mM glyphosate was applied, and 56–60% at doses of 0.1 and 0.2 mM, respectively.

During the 14-day exposure of radish roots to glyphosate, anthocyanin content increased slightly in the cotyledons of control radish seedlings. The use of glyphosate in a nutrient medium had no significant effect on anthocyanin content in the cotyledons after 4 and 7 days of exposure to the herbicide (fig. 1). However, after 14 days of exposure, there was a sharp increase in the content of that dye. At that time, the level of anthocyanins was 2.5 to 3 times higher than in the cotyledons of control seedlings.

In opposition to cotyledons, the anthocyanins content in the hypocotyl of control seedlings increased from 290 $\mu g \cdot g^{-1}$ fresh weight after 4-day of experiment to 640 $\mu g \cdot g^{-1}$ after 14 days (fig. 2). In this case, the four-day exposure to glyphosate did not affect the content of anthocyanins in the hypocotyl, and seven-day treatment reduced the level of anthocyanins in the highest concentration of glyphosate. However, the longer, 14-day exposure markedly decreased anthocyanins level which was twice lower under 2 mM concentration of herbicide as compared to the controlled organ.

Table 1. The impact of glyphosate added to nutrient medium and time of exposure on the length of root and height of shoot and whole radish seedlings

Object	Time of exposure (days)		
	4	7	14
	Shoot (mm)		
Control	31.2 b	39.3 a	35.4 a
Glyphosate 0.1 mM	35.7 a (114)	38.3 ab (97)	32.2 a (91)
Glyphosate 0.2 mM	37.4 a (120)	36.0 abc (92)	37.8 a (107)
Glyphosate 2.0 mM	31.4 b (100)	34.7 c (88)	34.4 a (97)
	Root (mm)		
Control	52.2 a	100.0 a	217.5 a
Glyphosate 0.1 mM	50.8 a (97)	69.0 ab (69)	52.6 b (24)
Glyphosate 0.2 mM	52.5 a (101)	52.4 b (52)	46.5 b (21)
Glyphosate 2.0 mM	38.8 b (74)	38.6 c (39)	34.8 c (16)
	Seedling (mm)		
Control	84.2 a	134.3 a	305.8 a
Glyphosate 0.1 mM	86.4 a (103)	114.3 a (85)	76.8 b (25)
Glyphosate 0.2 mM	89.2 a (106)	93.2 a (69)	80.4 b (26)
Glyphosate 2.0 mM	69.4 b (82)	73.3 b (55)	69.2 b (23)

Means in columns followed by the same letter are not significantly different at the $P \leq 0.05$ level as determined by Newman-Keuls test. In the parentheses are shown the value percentages as compared to control (100)

Table 2. The impact of glyphosate added to nutrient medium and time of exposure on the fresh mass of organs of radish seedlings

Object	Time of exposure (days)		
	4	7	14
	Cotyledons (mg)		
Control	55.3 ab	81.0 a	83.6 a
Glyphosate 0.1 mM	70.9 a (128)	70.4 ab (87)	57.8 b (69)
Glyphosate 0.2 mM	58.0 b (105)	64.2 b (79)	61.6 b (74)
Glyphosate 2.0 mM	50.4 b (91)	56.3 b (70)	38.4 c (46)
	Hypocotyl (mg)		
Control	30.6 b	40.3 a	36.4 a
Glyphosate 0.1 mM	39.3 a (128)	36.0 ab (89)	28.3 a (78)
Glyphosate 0.2 mM	35.8 b (117)	33.6 b (83)	35.9 a (84)
Glyphosate 2.0 mM	32.5 b (106)	31.3 b (78)	30.9 a (85)
	Roots (mg)		
Control	45.2 a	78.7 a	91.3 a
Glyphosate 0.1 mM	51.0 a (113)	59.4 ab (75)	50.7 b (56)
Glyphosate 0.2 mM	58.4 a (129)	50.4 bc (64)	54.7 b (60)
Glyphosate 2.0 mM	40.7 a (90)	39.8 c (51)	36.0 c (39)

Means in columns followed by the same letter are not significantly different at the $P \leq 0.05$ level as determined by Newman-Keuls test. In the parentheses are shown the value percentages as compared to control (100)

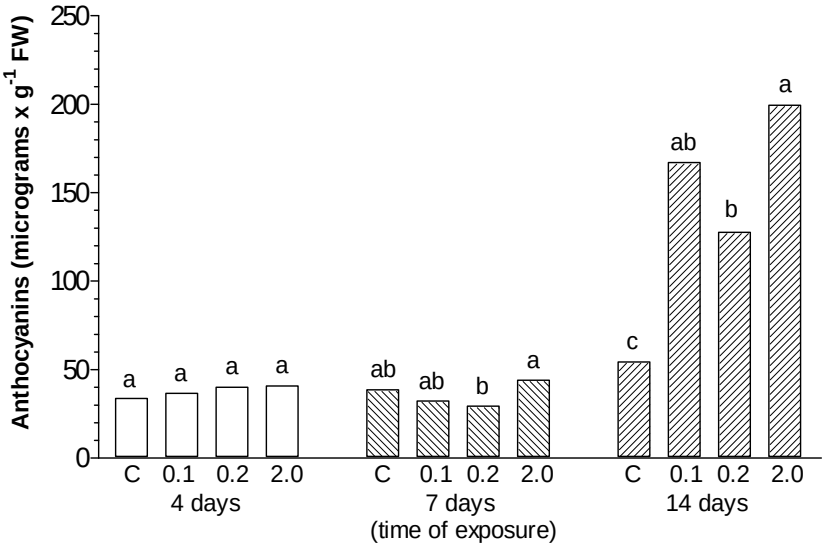

Fig. 1. The impact of glyphosate added to nutrient medium and time of exposure on the anthocynins content in the cotyledons of radish seedlings (C – control, no glyphosate; 0.1, 0.2, and 2.0 – concentration of glyphosate, mM). Mean results (bars) marked by the same letter are not significantly different at the $P \leq 0.05$ level as determined by Newman-Keuls test. The statistical analyses were carried out for 4, 7 and 14 days separately

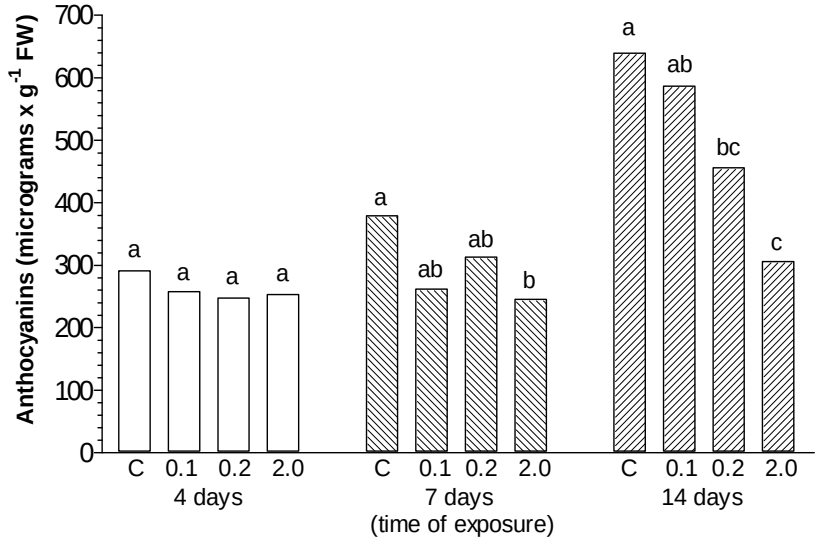

Fig. 2. The impact of glyphosate added to nutrient medium and time of exposure on the anthocyanins content in the hypocotyl of radish seedlings (C – control, no glyphosate; 0.1, 0.2, and 2.0 – concentration of glyphosate, mM). Mean results (bars) marked by the same letter are not significantly different at the $P \leq 0.05$ level as determined by Newman-Keuls test. The statistical analyses were carried out for 4, 7 and 14 days separately

DISCUSSION

The results obtained in our study indicate that glyphosate applied to the root zone has a different effect on the roots and above-ground parts of radish seedlings. Plants grown in a hydroponic medium containing glyphosate can take up the herbicide through the roots [Wagner et al. 2003, Saunders et al. 2013] which is transported mainly to the shoot apex [Alister et al. 2005]. However, only small amounts of the herbicide are available via this way [Giesy et al. 2000, Wagner et al. 2003, Dill et al. 2010]. Such low doses of glyphosate cause a hormetic effect in plants [Duke et al. 2006, Brito et al. 2018]. The results of our study show that short-term glyphosate treatment stimulated the shoot growth and biomass accumulation in the radish seedlings. The stimulation was observed only when low concentrations of herbicide were used. This is probably due to the fact that such concentration and treatment time cause a hormetic effect. What dose of glyphosate is taken by plant which cause such effects will be the subject of our further research.

In our experiments, glyphosate in nutrient medium had considerably higher impact on the growth of the primary root than shoot of the radish seedlings. These results confirm earlier findings in which it was shown that glyphosate has higher inhibitory effect on root growth compared to shoot [Cornish 1992, Lejczak et al. 1996, Petersen et al. 2007, Piotrowicz-Cieślak et al. 2010]. Roundup applied to root-zone decreased fresh weight of cucumber seedlings which did not exceed 20% compared to the control plants [Sacała et al. 2011]. The inhibitory effect of glyphosate occurring in the soil on growth of non-target plants was also demonstrated in sunflower seedlings [Tesfamariam et al. 2009].

Results of our study indicate that glyphosate present in nutrient medium resulted in the decrease of anthocyanins content in the hypocotyls and its increase in the cotyledons after longer exposure. An inhibition of the anthocyanins accumulation by glyphosate has been generally known because this is the basic effect of glyphosate which causes phenylpropanoid biosynthesis disturbances in plants [Amrhein et al. 1980, Hoagland 1980, Lejczak et al. 1996]. The herbicide inhibits the shikimate pathway, which blocks the synthesis of aromatic amino acids and many secondary metabolites, also such as anthocyanins [Duke et al. 2012]. However, results of our study provide new information. Glyphosate inhibited the accumulation of anthocyanins in the hypocotyl of radish seedlings, but not in cotyledons. It seems that the reason for this is the low amount of glyphosate absorbed by the roots and transported to cotyledons which are not able to inhibit the biosynthesis of anthocyanins. Such doses of the herbicide only led to oxidative stress which results in the increased accumulation of the anthocyanins. This hypothesis, however, requires further research.

CONCLUSIONS

1. Short-term exposure of the radish roots to low doses of glyphosate leads to a stimulation of growth and biomass accumulation and has no effect on the contents of anthocyanins in the cotyledons and hypocotyls.

2. Prolonged exposure of radish roots to glyphosate causes a marked inhibition of the growth of roots and shoots of the seedling. This has been accompanied by enhanced accumulation of anthocyanins in the cotyledons and their reduction in hypocotyls.

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REFERENCES

Alister, C., Kogan, M., Pino, I. (2005). Differential phytotoxicity of glyphosate in maize seedlings following applications to roots or shoot. Weed Res., 45, 27–32.

Amrhein, N., Deus, B., Gehrke, P., Steinrücken, H.C. (1980). 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., 66, 830–834.

Baig, M.N., Darwent, A.L., Harker, K.N., O'Donovan, J.T. (2003). Preharvest applications of glyphosate affect emergence and seedling growth of field pea (*Pisum sativum*). Weed Technol., 17, 655–665.

Borggaard, O.K., Gimsing, A.L. (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: A review. Pest Manag. Sci., 64, 441–456.

Banaszkiewicz, T., Wysocki, K. (2012). Application of white mustard (*Sinapis alba*) biotest in the assessment of environmental contamination by glyphosate. Pol. J. Environ. Stud., 21, 1161–1166.

Basantani, M., Srivastava, A., Sen, S. (2011). Elevated antioxidant response and induction of tau-class glutathione S-transferase after glyphosate treatment in *Vigna radiata* (L.) Wilczek. Pest. Biochem. Physiol., 99, 111–117.

Bott, S., Tesfamariam, T., Kania, A., Eman, B., Aslan, N., Römheld, V., Neumann, G. (2011). Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilisation. Plant Soil, 342, 249–263.

Brito, I.P., Tropaldi, L., Carbonari, C.A., Velini, E.D. (2017). Hormetic effects of glyphosate on plants. Pest Manag. Sci., 74, 1064–1070. DOI: 10.1002/ps.4523.

Clay, P.A., Griffin, J.L. (2000). Weed seed production and seedling emergence responses to late-season glyphosate applications. Weed Sci., 48, 481–486.

Cornish, P.S. (1992). Glyphosate residues in a sandy soil affect tomato transplants. Austr. J. Exp. Agric., 32, 395–399.

Dill, G.M., Sammons, R.D., Feng, P.C.C., Kohn, F., Kretzmer, K., Mehrsheikh, A., Bleeke, M., Honegger, J.L., Farmer, D., Wright, D., Haupfear, E.A. (2010). Glyphosate: discovery, development, applications, and properties. In: Glyphosate resistance in crops and weeds: history, development, and management. Nandula, V.K. (ed.). John Wiley and Sons, Hoboken, 1–33.

Duke, S.O., Cedergreen, N., Velini, E.D., Belz, R.G. (2006). Hormesis: is it an important factor in herbicide use and allelopathy. Outlooks Pest. Manag., 17, 29–33.

Duke, S.O., Lydon, J., Koskinen, W.C., Moorman, T.B., Chaney, R.L., Hammerschmidt, R. (2012). Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. J. Agric. Food Chem., 60, 10375–10397.

Ellis, J.M., Griffin, J.L. (2002). Soybean (*Glycine max*) and cotton (*Gossypium hirsutum*) response to simulated drift of glyphosate and glufosinate. Weed Technol., 16, 580–586.

Giesy, J.P., Dobson, S., Solomon, K.R. (2000). Ecotoxicological risk assessment for Roundup® herbicide. In: Reviews of environmental contamination and toxicology. Communicated, Ware, G.W. (ed.). Springer, New York, 35–120.

Hetherington, P.R., Reynolds, T.L., Marshall, G., Kirkwood, R.C. (1999). The absorption, translocation and distribution of the herbicide glyphosate in maize expressing the CP-4 transgene. J. Exp. Bot., 50, 1567–1576.

Hoagland, R.E. (1980). Effects of glyphosate on metabolism of phenolic compounds. VI. Effects of glyphosine and glyphosate metabolites on phenylalanine ammonia-lyase activity, growth, and protein, chlorophyll, and anthocyanin levels in soybean (*Glycine max)* seedlings. Weed Sci., 28, 393–400.

Komives, T., Schroder, P. (2016). On glyphosate. Ecocycles, 2, 1–8.

Lejczak, B., Boduszek, B., Kafarski, P., Forlani, G., Wojtasek, H., Wieczorek, P. (1996). Mode of action of herbicidal derivatives of aminomethylenebisphosphonic acid. I. Physiologic activity and inhibition of anthocyanin biosynthesis. J. Plant Growth. Regul., 15, 109–113.

Liu, L., Punja, Z.K., Rahe, J.E. (1997). Altered root exudation and suppression of induced lignification as mechanisms of predisposition by glyphosate of bean roots (*Phaseolus vulgaris* L.) to colonization by *Pythium* spp. Physiol. Mol. Plant Pathol., 51, 111–127.

Mancinelli, A.L. (1984). Photoregulation of anthocyanin synthesis. VIII. Effects of light pretreatments. Plant Physiol., 75, 447–453.

Mateos-Naranjo, E., Perez-Martin, A. (2013). Effects of sub-lethal glyphosate concentrations on growth and photosynthetic performance of non-target species *Bolboschoenus maritimus*. Chemosphere, 93, 2631–2638.

Petersen, I.L., Hansen, H.C., Ravn, H.W., Sørensen, J.C., Sørensen, H. (2007). Metabolic effects in rapeseed (*Brassica napus* L.) seedlings after root exposure to glyphosate. Pest. Biochem. Physiol., 89, 220–229.

Piotrowicz-Cieślak, A.I., Adomas, B., Michalczyk, D.J. (2010). Different glyphosate phytotoxicity of seeds and seedlings of selected plant species. Pol. J. Environ. Stud., 19, 123–129.

Sacała, E., Demczuk, A., Grzyś, E. (2011). Laboratory study to investigate the response of *Cucumis sati-*

vus L. to Roundup and Basta applied to the rooting medium. In: Herbicides – mechanisms and mode of action, El-Ghany Hasaneen, M.N.A. (ed.). Chapter 3. InTech, Rijeka, http://www.intechopen.com/books/herbicides-mechanisms-and-mode-of-action, 49–62.

Satchivi, N.M., Wax, L.M., Stoller, E.W., Briskin, D.P. (2000). Absorption and translocation of glyphosate isopropylamine and trimethylsulfonium salts in *Abutilon theophrasti* and *Setaria faberi*. Weed Sci., 48, 675–679.

Saunders, L.E., Koontz, M.B., Pezeshki, R. (2013). Root-zone glyphosate exposure adversely affects two ditch species. Biology, 2, 1488–1496.

Silva, F.B., Costa, A.C., Alves, R.R.P., Megguer, C.A. (2014). Chlorophyll fluorescence as an indicator of cellular damage by glyphosate herbicide in *Raphanus sativus* L. plants. Amer. J. Plant Sci., 5, 2509–2519.

Tesfamariam, T., Bott, S., Cakmak, I., Römheld, V., Neumann, G. (2009). Glyphosate in the rhizosphere – Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants. Eur. J. Agron., 31, 126–132.

Velini, E.D., Alves, E., Godoy, M.C., Meschede, D.K., Souza, R.T., Duke, S.O. (2008). Glyphosate applied at low doses can stimulate plant growth. Pest Manag. Sci., 64, 489–496.

Wagner, R., Kogan, M., Parada, A.M. (2003). Phytotoxic activity of root absorbed glyphosate in corn seedlings (*Zea mays* L.). Weed Biol. Manag., 3, 228–232.