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Laboratory Study to Investigate the Response of *Cucumis sativus* L. to Roundup and Basta Applied to the Rooting Medium

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1. Introduction

Roundup and Basta are commonly used herbicides in both agricultural and non-agricultural systems. They are non-selective broad spectrum post-emergence herbicides, relatively non-toxic to environment. Their mode of action is connected with inhibition of amino acids biosynthesis (Cobb, 1992; Wakabayashi & Böger, 2004). Herbicide Basta is also know as glufosinate or phosphinothricin. Glufosinate is a structural analogue of glutamic acid and it irreversibly inhibits glutamine synthetase (GS) which synthesises glutamine from glutamate and ammonium. Consequently, ammonium concentration in plant tissues strongly increases and causes metabolic disruption and plants' death. Roundup's active ingredient is glyphosate [N-(phosphonomethyl)glycine] that inhibits 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS). As a consequence, the biosynthesis of aromatic amino acids is inhibited. Besides, glyphosate is known to have secondary mode of action connected with the inhibition of chlorophyll biosynthesis resulting from a glyphosate-mediated inhibition of δ-aminolevulinic acid synthesis (Pline et al., 1999). Both herbicides alter the cellular pools of amino acids that results in disruption of nitrogen metabolism. Indirectly they affect protein synthesis, nitrate assimilation and nitrogen fixation (Bellaloui et al., 2006; Sacała et al., 2008). Nitrogen is an essential element that strongly influence and regulate plants' growth. For most plants in moderate climate regions, the main source of nitrogen is nitrate obtained from the soil. Nitrate is utilized in a linear pathway that involves its uptake and transport within the plant and then its assimilation, ammonium assimilation and amino acid and protein biosynthesis (Sitt et al., 2002). The first step in nitrate assimilation pathway, the reduction of nitrate to nitrite, is catalyzed by nitrate reductase (NR, EC 1.6.6.1–2). This enzyme plays a central role in nitrogen assimilation and is considered as a key point of metabolic regulation, as well as a rate-limiting enzyme in this pathway. Nitrate reductase is known to modulate rapidly to environmental stress conditions and is responsive to metabolic and physiological status of plants (Kaiser & Huber, 1994). Hence, sometime it is used as a biomarker of plant stress. Nitrate reduction occurs in both shoots and roots of plants and the relative contribution of each is dependent on the particular species, plant age and growth conditions.

Basta and Roundup are taken up by plants after application on the leaves and then are transported throughout the whole plant. Phytotoxicity of both herbicides is certainly rapid and leaf chlorosis, desiccation and necrosis may be observed within few days after application (Cobb, 1992).

In general opinion, Basta and Roundup don't accumulate in the environment and rapidly break down. However, there are reports showing that the half-life values of glyphosate vary on a wide range from a few days to several months and even years (Vereecken , 2005). Herbicide contamination in the soil may originate from foliar washing off, undirected spray drift, exudation from roots, and death and decomposition of treated plant residues (Hanke et al., 2010; Tesfamariam et al., 2009). Glyphosate is often found in surface waters and rarer in groundwater. For this reason, there is a risk that contamination of herbicide in soil may have detrimental effect on non-target plants.

The purpose of this study was to evaluate the effect of relatively low, non-lethal concentration of glyphosate and glufosinate, applied directly to root zone, on young fast growing cucumber seedlings. The concentrations of herbicides chosen in the assessment were not the same because a negative impact of examined herbicides on the environment and a risk of pollution are substantially different. Roundup is one of the most widely used herbicide in the world and its application is increasing all the time. This increase is due to a widespread cultivation of glyphosate-resistant crops (e.g. cotton, canola, corn, alfalfa, sugar beet). Hence, an expected amount of Roundup residues in soil is higher than that of Basta (Service, 2007; Woodburn 2000).

2. Experimental procedures

2.1 Plant material and growth conditions

The experiments were conducted on cucumber seedlings (*Cucumis sativus* L. var. Władko F-1) grown in hydroponic cultures. The experimental design consisted of following treatments: (i) control – 0.33-strength Hoagland nutrient solution, (ii) glyphosate treatment – 0.33-strength Hoagland nutrient solution plus Roundup herbicide in formulation 360 g a.i. per litre (at the concentration of 22 $\mu l \cdot litre^{-1}$ of nutrient solution that corresponds to 0.0467 mM glyphosate), (iii) glufosinate treatment – control plus Basta herbicide in formulation 150 g a.i. per litre (at the concentration of 0.0077 mM glufosinate). Culture conditions were as follows: 16 h photoperiod (220 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 26/20°C day/night, 65-70% relative humidity.

2.2 Plant analyses

After 7 days of cultivation there were investigated growth parameters (length, fresh and dry weight of roots and shoots), activity of nitrate reductase (NRA) and concentrations of photosynthetic pigments and protein (soluble, insoluble and total).

2.2.1 Plant growth analysis

After 7 days of cultivation, plants were harvested and separated into roots and shoots and their lengths were measured. After that, plant organs (separately roots, hypocotyls and cotyledons) were weighted and dried at 105°C for 1 h and subsequently at 75°C. Then the dry weight was determined.

2.2.2 Determination of water content

The water content (WC) in roots and shoots was calculated on a fresh weight (FW) basis. WC was computed as the difference between the fresh weight and the dry weight divided by the fresh weight and multiplied by 100%.

2.2.3 Biochemical analyses

2.2.3.1 Photosynthetic pigments

Photosynthetic pigments were extracted from cotyledons using 80% acetone. Absorbance of obtained extracts was recorded at 470, 647, 663 nm and concentration of chlorophyll a, chlorophyll b, total chlorophyll (chl a + chl b) and carotenoids were calculated using the equations of Lichtenthaler (1987).

2.2.3.2 Protein concentration

For protein determination, 0.5 g of roots or cotyledons was homogenised with 0.1 M phosphate buffer (0.1 M K_2HPO_4-KH_2PO_4, pH 7.5 contained 1 mM EDTA) and then homogenate was centrifuged at 4°C (12000×g, 10 min). After centrifugation both the supernatant and the precipitate were collected. Soluble protein was measured in the supernatant according to the Lowry method (Lowry et al., 1951). Insoluble protein in the precipitate was extracted with 0.1 M NaOH at 60°C for 40 min and then the sample was centrifuged (15000×g, 10 min) and in supernatant protein was measured by the Lowry method.

2.2.3.3 Nitrate reductase activity (NRA)

Nitrate reductase activity was determined *in vivo* method according to Jaworski (1971). The plant material (1 g) was cut into 3 mm segments and placed in test-vial containing 20 cm^3 phosphate buffer (0.1 M K_2HPO_4-KH_2PO_4, pH 7.5 contained 100 mM KNO_3 and 0.5% isopropanol). The test-vials were vacuum infiltrated for 2 min and then incubated in the dark at 25°C for 1 h. The amount of nitrite formed during the reaction was measured spectrophotometrically at 540 nm after adding 1% sulfanilamide in 0.1 M HCl and 0.02% N-naphtyl-ethylenediamine.

2.3 Statistical analysis

The experiment was arranged in a randomized complete block design and was repeated six times. The data for all parameters were statistically analysed using the variance analysis and the differences among mean values were compared by the least significant difference test (LSD, $P \leq 0.05$).

3. Results

3.1 Growth parameters and water content

All plants survived under examined conditions, however both herbicides caused significant reduction in fresh and dry weights of the above- and underground parts of plants (Fig. 1 A, B, C).

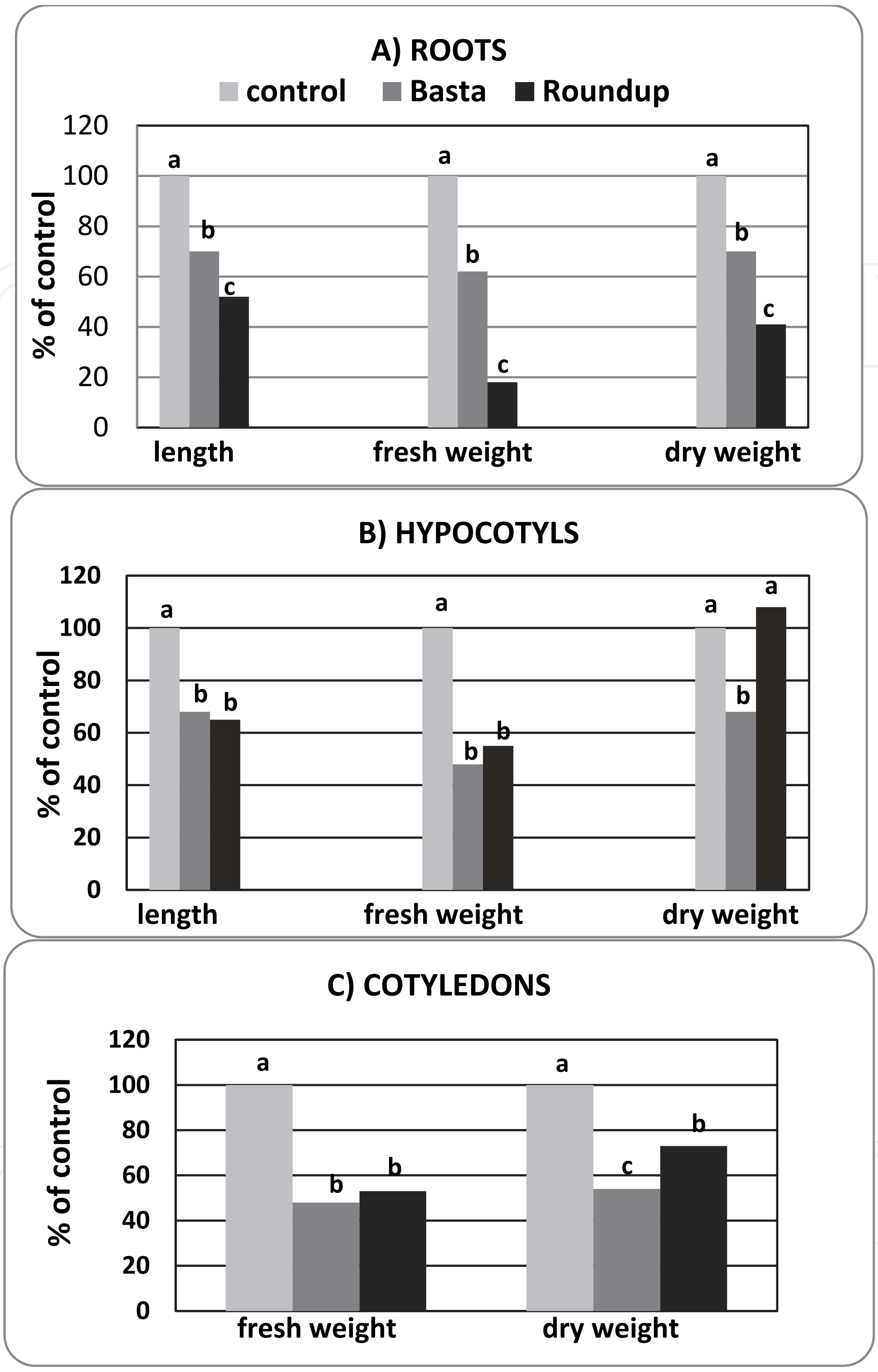

Fig. 1. The influence of Basta and Roundup on growth parameters of 7-day old cucumber seedlings. Values with the same letter above the bars do not differ significantly at P≤0.05.

The exception were hypocotyls of plants treated by Roundup. Their dry weight was similar to the control plants in contrast to fresh weight that was reduced by 45% comparing to non-treated plants. Roundup caused dramatic decrease in fresh weight of cucumber roots and this parameter lowered to 18% of the control value (Fig. 1A). Length of roots and their dry weight were also significantly reduced by Roundup treatment but this reduction did not exceed 60%. Basta also markedly inhibited growth of cucumber roots but this inhibition ranged from 30% for roots' length and dry weight to 38% for fresh weight. Basta and Roundup similarly affected height and fresh weight of hypocotyls whereas in dry matter accumulation there was difference (Fig. 1B). Basta caused 32% decrease in this parameter comparing to the control plants while Roundup did not act negatively. Both herbicides caused similar decrease in fresh weight of cotyledons (approximately 50% compared to the control plants) but Basta more reduced dry matter accumulation than Roundup (Fig. 1C).

Exposure of maize seedlings to herbicides had significant effect on water content in plants' tissues (Tab. 1).

	Water content (% of FW)		
	Control	Basta	Roundup
Roots	96.68±0.12 a	96.51±0.13 a	93.72±0.41 b
$LSD_{0.05}$	0.41		
Hypocotyls	96.46±0.26 a	95.49±0.38 b	92.08±0.20 c
$LSD_{0.05}$	0.46		
Cotyledons	92.81±0.17 a	92.07±0.27 b	89.21±0.24 c
$LSD_{0.05}$	0.37		

Table 1. The influence of Basta and Roundup on water content in different organs of 7-day old cucumber seedlings. Values in the same row followed by the same letter do not differ significantly at $P \leq 0.05$.

Roundup caused significant decrease in water content in all cucumber organs and the highest decline occurred in hypocotyls and it amounted to above 4% in comparison to the control plants. In cotyledons and roots, recorded decreases were respectively 3.60 and 2.96% compared to non-treated plants. Basta at examined concentration did not change significantly water content in cucumber roots but lowered this parameter significantly in hypocotyls and cotyledons (Tab. 1). However, observed changes in water content were considerably lower than under Roundup treatment (they did not exceed 1% comparing to the control plants). Hence, Roundup more effectively than Basta (in tested concentration) acted as a plant desiccant.

3.2 Photosynthetic pigments

Cotyledons of cucumber seedlings growing in nutrient medium contained herbicides were dark green. Chlorophyll concentration in these cotyledons was significantly higher than in the control plants (Tab. 2). Exposure of seedlings to Basta herbicide resulted in 8% increase in all forms of chlorophyll, while Roundup caused approximately 12% increase. Moreover, chlorophyll a to chlorophyll b ratio (Chl a/Chl b) was not affected and this value amounted about 3.0 under all treatments. Nevertheless, there were recorded differences in values of total chlorophylls to carotenoids ratio (Tab. 2). Under herbicides treatment this value showed tendency to lowering compared to non-treated plants. This change results from relatively higher increase in carotenoids contents comparing to increased level of chlorophylls. Basta and Roundup caused respectively 14 and 23% increase in carotenoids concentration comparing to the control plants.

	Control	Basta	Roundup
Chl a [$\mu g \cdot g^{-1}$ FW]	1134.2±37.5 b (100%)	1224.8±62.2 a (108%)	1267.8±8.2 a (112%)
$LSD_{0.05}$		63.3	
Chl b [$\mu g \cdot g^{-1}$ FW]	379.6±15.4 b (100%)	408.1±10.3 a (108%)	420.4±3.3 a (111%)
$LSD_{0.05}$		22.5	
Total Chl [$\mu g \cdot g^{-1}$ FW]	1513.8±51.5 b (100%)	1632.9±80.7a (108%)	1688.1±53.0 a (112%)
$LSD_{0.05}$		99.3	
Carotenoids [$\mu g \cdot g^{-1}$ FW]	239.1±2.7c (100%)	271.7±13.8 b (114%)	293.7±16.9 a (123%)
$LSD_{0.05}$		19.1	
Chl a/Chl b	2.99	3.00	3.02
Total Chl/Car	6.33	6.01	5.75

Table 2. Photosynthetic pigments concentration in cotyledons of 7-day old cucumber seedlings. Values in the same raw marked with the same letters don't differ significantly at $P \leq 0.05$. Values in parentheses indicate the percent of control.

3.3 Protein concentration

Protein concentration in roots and cotyledons was affected differentially by examined herbicides (Tab. 3). Roundup caused significant increase in the protein concentration in both roots and cotyledons and in roots the total protein content increased by 42% in comparison to the control plants (Tab. 3). There was observed huge increase in soluble fraction of protein and it amounted to 166% of the value in the control plants. In cotyledons protein content was not such strongly affected as in roots, nevertheless insoluble fraction of protein was higher (15% increase) than in non-treated plants. Under Basta application there was observed a tendency to lowering protein concentration but the differences were not statistically significant.

3.4 Nitrate reductase activity (NRA)

Nitrate reductase activity was dramatically inhibited in cotyledons of cucumber growing in nutrient solution contained Roundup (Tab. 4). Enzyme activity lowered to 3% of the value in control plants. Basta application also caused decrease in enzyme activity (39% reduction) but it was relatively small compared to Roundup treatment. Both Basta and Roundup caused similar (approximately 2-fold) increase in nitrate reductase activity in cucumber roots.

	Protein concentration [mg·g^{-1} FW]		
	Control	Basta	Roundup
Roots			
Soluble protein	5.25±0.27 b (100%)	5.50±0.35 b (105%)	8.71±0.91 a (166%)
$LSD_{0.05}$	0.80		
Insoluble protein	4.76±0.46 b (100%)	4.20±0.53 b (88%)	5.51±0.48 a (116%)
$LSD_{0.05}$	0.70		
Total protein	10.01±0.55 b (100%)	9.70±0.58 b (97%)	14.22±0.90 a (142%)
$LSD_{0.05}$	1.06		
Cotyledons			
Soluble protein	19.60±1.19 a (100%)	19.64±1.29 a (100%)	21.70±2.07 a (111%)
$LSD_{0.05}$	2.70		
Insoluble protein	11.55±0.91 b (100%)	10.15±0.67 b (88%)	13.30±1.22 a (115%)
$LSD_{0.05}$	1.54		
Total protein	31.15±1.37 b (100%)	29.75±1.79 b (96%)	35.00±2.56 a (112%)
$LSD_{0.05}$	3.22		

Table 3. Protein concentration in roots and cotyledons of 7-day old cucumber seedlings. Values in the same raw marked with the same letters don’t differ significantly at $P \leq 0.05$. Values in parentheses indicate the percent of control.

	NRA [nmol(NO_2^-) ·g^{-1} FW ·h^{-1}]		
	Control	Basta	Roundup
Roots	111.0±19.7 b (100%)	201.2±32.5 a (181%)	233.1±37.6 a (210%)
$LSD_{0.05}$	40.2		
Cotyledons	847.2±116.6 a (100%)	515.0±108.1 b (61%)	29.7±6.7 c (3%)
$LSD_{0.05}$	122.4		

Table 4. Nitrate reductase activity in roots and cotyledons of 7-day old cucumber seedlings. Values in the same raw marked with the same letters don’t differ significantly at $P \leq 0.05$. Values in parentheses indicate the percent of control.

4. Discussion

Herbicides are increasingly used in both agricultural and non-agricultural system as a quick, easy and inexpensive remedy for weeds control. There are many compounds registered as herbicides, which may be classified according to their mode of action. Very important and popular set of herbicides are that interfering with amino acid biosynthesis. They inhibit particular enzymes in plants and consequently block amino acid synthesis (Cobb, 1992; Tan et al., 2006). There are three major enzymes that can be inhibited: (1) acetolactate synthase (AHAS, EC 4.1.3.18) involved in the branched-chain amino acid biosynthesis pathway; (2) synthase of 5-enolpyruvyl shikimate-3-phosphate (EPSPS, EC 2.5.1.19) operating in the shikimate pathway and biosynthesis of aromatic amino acids; (3) glutamine synthetase (GS, EC 6.3.1.2) the key enzyme in ammonium assimilation. In our study we focused on chemicals belonging to two later classes. These are glyphosate, the active ingredient of Roundup herbicide, and glufosinate, the active compound of Basta herbicide. Both chosen herbicides are extensively used worldwide and show some similarities. They are systemic, non-selective, broad spectrum post-emergence herbicides, relatively non-toxic to environment. Moreover, for both herbicides there have been developed herbicide-resistant plants but glyphosate-resistant crops dominate on the world (Service, 2007). Cultivation of herbicide-resistant plants results in the expanded use of these non-selective herbicides. Hence, there is an increasing concern regarding the potential risk of herbicides' pollution and their toxicity to non-target organisms (Blacburn & Boutin, 2003; Boutin et al., 2004; Tesfamariam et al., 2009). The researches concerning the potential phytotoxicity of herbicides to non-target higher plants examine two main aspects of this problem. On the one side, there is investigated the influence of simulated spray drift of herbicides on non-target plants. On the other side, the investigations are conducted to determine the effect of soil applied herbicides (the residues of herbicibes in soil) on plants. In our study we investigated the latter aspect. Herbicides can reach the soil via foliar wash off, undirected spray drift contamination and decomposition of treated plant residues (Tesfamariam et al., 2009). Besides, increased glyphosate concentration in soil may originate from plant roots and exuding process (Laitinen et al., 2007). For our experiment we chose cucumber (*Cucumis sativus* L.), that is very important and popular species in horticultural production. Both herbicides caused visible inhibition of plant growth but there were not observed such injury symptoms as chlorosis or necrosis. Nevertheless, shoots of cucumber exposed to Roundup showed marked symptoms of wilting. Basta and Roundup similarly affected height and fresh weight of hypocotyls and fresh weight of cotyledons. Cucumber roots were very sensitive to Roundup and their fresh weight did not exceed 20% compared to the control plants and was nearly 4-fold lower (on the fresh weight basis) than under Basta treatment. For both herbicides and all organs (roots, hypocotyls, cotyledons) a percentage inhibition in fresh matter accumulation was higher than that in dry matter (Fig. 1A, B, C). This evidently indicates that both Basta and Roundup present in rooting medium markedly disrupt water relations in cucumber tissues. Results in Tab. 1 also show that water status of cucumber organs was strongly impaired, particularly in seedlings exposed to Roundup. These results indicate that the observed inhibition of plant growth could be, in part, caused by the disturbances in water status in cucumber tissues. Negative effect of soil-applied glyphosate and glufosinate on growth of non-target plants was also observed in experiments on sunflower, tomato, maize and cucumber seedlings (Sacała et al., 1999; Sacała et al., 2008;

Tesfamariam et al., 2009; You & Barker, 2005). The most literature data show that application of glufosinate and glyphosate resulted in a decrease in chlorophyll content in plants (Cakmak et al., 2009, Kielak et al., 2011; Pline et al., 1999; Reddy et al., 2000; Zaidi et al., 2005). Our results don't agree with those mentioned above. Cotyledons of cucumber seedlings exposed to herbicides were dark-green and contained more both chlorophyll and carotenoids than non-treated plants (Tab. 2). This indicates that at early stage of cucumber growth (the stadium of fully developped cotyledons) synthesis of chlorophyll is not inhibited by examined herbicides and the ratio of chlorophyll a to chlorophyll b is not impaired. Additionally, increase in total pool of carotenoids in cucumber cotyledons could be an adaptive feature preventing photooxidative damages in chloroplasts. Some researchers express a view that a loss of chlorophyll under stress conditions may be a positive symptom preventing photoinhibition of the photosynthetic apparatus (Maslova & Popova 1993). As mentioned in the Introduction, secondary phytotoxic effect caused by glyphosate is connected with the inhibition of the porphyrin precursor synthesis - δ-aminolevulinic acid – that results in an inhibition in chlorophyll biosynthesis and visible symptoms of chlorosis (Pline et al., 1999). Our results showed that glyphosate applied to the rooting medium did not disturb chlorophyll synthesis in cotyledons but stopped its biosynthesis in leaf. It is worth to note, that the first hardly emerged leaf of cucumber was completely yellow. Moldes et al. (2008) maintain that plants with low constitutive level of chlorophyll might have slower reduction in chlorophyll synthesis rate induced by glyphosate. Whereas, Wong (2002) indicated that the reduction in chlorophyll a content may be dependent on glyphosate concentration and concentration of 2 mg/l or more caused a significant decrease in the level of chlorophyll a.

Both examined herbicides disrupt nitrogen metabolism. The primary disruptions caused by these herbicides take place at different points of the nitrogen assimilation pathway but there may also appear secondary effects connected with the impairment of protein synthesis and functioning of others enzymes involved in nitrate assimilation. Hence, in our study we assumed that nitrate reductase activity and protein concentration will be the common indices of disturbances in nitrogen metabolism caused by both Roundup and Basta. Nitrate reduction in plant tissues is a fundamental process in nitrogen assimilation. The first reaction in nitrate reduction is catalyzed by nitrate reductase. Activity of this enzyme is precisely regulated and controlled by different internal and environmental factors. Among the internal factors closely connected with nitrogen metabolism there are: availability of substrate NO_3^-, concentration of ammonium and the level of end products of nitrate assimilation, mainly glutamine and glutamate (Tischner, 2000). Nitrate stimulates nitrate reductase activity, whereas ammonium and nitrogen compounds accumulated in plants' tissues may repress nitrate assimilation. Reddy et al. (2010) examined the influence of glyphosate simulated drift effect on non-glyphosate-resistant corn. They indicated that glyphosate drift significantly reduced both leaf nitrogen and nitrate reductase activity. Maximum reduction in nitrate reductase activity amounted to 64% compared to non-treated plants. Similar results were obtained by Bellaloui et al. (2006) for non-glyphosate-resistant soybean. Moreover, these researchers indicated that nitrate reductase activity in soybean roots, opposite to the aboveground organs (leaves and stems), was not affected by glyphosate application. Additionally, when glyphosate was applied 6 weeks after planting there was observed large (approximately 2-fold) increase in enzyme activity compared to the untreated control plants. We obtained similar results and we observed nearly 2-fold increase in nitrate reductase activity in cucumber roots growing in medium contained Roundup compared to control plants. In our previous study (Sacała et al., 1999) we also demonstrated that Roundup significantly increased nitrate reductase activity in roots of maize and cucumber var. Wisconsin compared to non-treated plants, whereas roots' growth was strongly suppressed. Basta also caused significant increase in nitrate reductase activity in roots and decrease in cotyledons but observed reduction was not such dramatic as in the case of Roundup application (Tab. 4). Residual nitrate reductase activity in cotyledons of cucumber exposed to glyphosate may result from a loss of enzyme protein (inhibition of enzyme synthesis and inactivation/degradation of already synthesized enzymes), and in part, from the shortage of available reductants for NO_3^- reduction (enzyme activity was assayed at the endogenous level of NADH in plant cells). Under Basta treatment decrease in nitrate reductase activity also could be caused by low availability of NADH. On the other side, it can be assumed that ammonium accumulated in plant cells was important factor repressing this enzyme. Inhibition of glutamine synthetase causes glutamine deficiency and excessive accumulation of ammonium in plant tissues (Pornprom, et al., 2003, Sacala et al., 2008; Wakabayashi & Böger, 2004). All these results show that nitrate reductase from plant roots is less sensitive to examined herbicides than that in cotyledons. Increase in nitrate reductase activity in roots of cucumber exposed to herbicide may be considered as an adaptive feature allowing to compensate a reduction in cotyledons. Bellaloui et al. (2006) also maintain that the assimilation of nitrate in the roots helps minimize the stress effect of herbicide on plant growth. But it is worth to note, that observed increase in enzyme activity, although relatively high, was too low to sustain nitrate reduction in the whole plant at a level similar to the control plants.

Both examined herbicides destroy biosynthesis of intrinsic amino acids and in turn may disrupt synthesis of protein and other N-containing compounds. It is very interesting that in cucumber seedlings, growing in medium contained herbicides, any decrease in protein concentration was not observed (Tab. 3). Basta did not change protein concentration in roots and cotyledons whereas Roundup markedly increased its amount. The largest increase was noticed in roots in the pool of soluble protein. In this case protein concentration was 166% of the value in the control plants. It can be assumed that in this pool are specific proteins defined as stress-associated proteins. Biosynthesis of these proteins may be induced by exposure to stress factors or they may be present constitutively at low level and are elevated in unfavourable conditions. This increased pool of protein might also include the enzymes involved in antioxidant defense mechanisms (Moldes et al., 2008). As mentioned above, Roundup significantly decreased water content in roots and simultaneously increased markedly nitrate reductase activity. Literature data state that nitrate reductase is characterized by high sensitivity to water deficit and its activity rapidly falls under water stress (Barathi, et al., 2001; Burman, et al. 2004). It is possible that some stress proteins protect root enzymes and their mRNA against negative influence of water deficit and directly against damage caused by Roundup. Concentration of insoluble protein also significantly increased although this rise was lower than in the case of soluble protein (16% compared to the control). These changes indicate that cucumber roots activate the biochemical mechanisms that may prevent damage caused by Roundup. It can be assumed that roots being directly exposed to herbicides increase protein synthesis, particularly its metabolically active fraction - soluble protein. However, synthesis of structural (insoluble) protein was also intensified. The response of cotyledons was slight different. Protein concentration in these organs also increased in comparison to non-treated plants but statistically significant increase was noticed in the fraction of insoluble protein and it was similar to that observed in cucumber roots. Proteins accumulated in plant tissues amongst several functions mentioned above may also serve as a storage form of nitrogen that can be utilized within a recovery of growth when stress is over.

Presented results show that relatively low concentrations of Roundup and Basta significantly inhibit growth of cucumber and cause profound changes in protein synthesis and nitrate reductase activity. The changes induced by Basta were lower than those caused by Roundup but concentration of Basta in nutrient solution was 6-fold lower (comparing the molal concentrations) than that of Roundup.

In light of the obtained results, as well as literature data about some weaknesses and risk of herbicide application (Baylis, 2000; Blackburn and Boutin, 2003; Laitinen et al., 2007) it can be concluded, that further increased application of herbicides may have strong stressing effect on non-target plants.

5. Conclusion

Obtained results show that small amount of the available herbicides' residues in root zone may effectively inhibit growth of non-target plants and significantly change their metabolism. Examined plant – cucumber at early phase of growth – is very sensitive to both herbicides but particularly to Roundup. Roundup considerably suppresses the growth of cucumber roots, lowers water content in all organs and dramatically decreases nitrate reductase activity in cotyledons.

The changes in biochemical parameters induced by Basta are smaller than that caused by Roundup.

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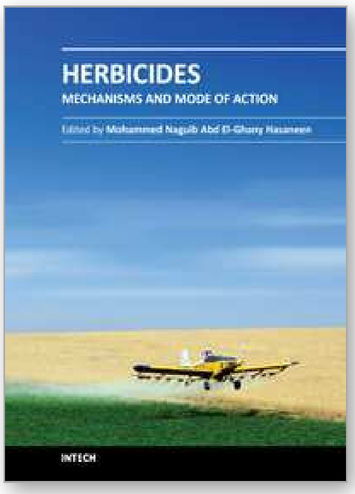

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This volume contains two sections: Mechanisms of herbicidal action (chapters 1-4) and Mode of action of selected herbicides on controlling diseased, weed growth and productivity and/or growth and development of field crops (chapters 5-10). Topics by chapters are: molecular mechanism of action, immunosensors , laboratory studies, molecular modeling, weed resistance, community response, use of herbicides in biotech culture, gene flow, herbicides and risk, herbicides persistence. These recurring themes reinforce my view, held over a very long time, that experience with one crop or problem can sometimes be relevant, often to an unexpected extent, to an apparently dissimilar situation in a different crop. I hope that readers interested in herbicides and pesticides will be satisfied with all the chapters in the book as its content might be of interest and value to them in the future.