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Abiotic stress affects the yield and nutrients of buckwheat grains

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Abstract

The accumulation of valuable nutrients in grains is dependent on a number of factors, including biotic and abiotic stress, and the effects of herbicides. The aim of this study was to determine the effect of a metazachlor and clomazone mixture and 2-methyl-4-chlorophenoxyacetic acid (MCPA) on the chemical composition of the neutral detergent fibre, lipids, protein and ash content of common buckwheat (*Fagopyrum esculentum* Moench) seeds. A field experiment was conducted in 2013–2015. The mixture containing metazachlor (750 g ha^{-1}) and clomazone (96 g ha^{-1}) applied one day after buckwheat sowing proved to be phytotoxic and caused leaf chlorosis. These symptoms persisted until the end of the flowering stage and gradually disappeared during the seed formation stage. MCPA applied at the stage of two true leaves at a dose of 750 g ha^{-1} showed a higher phytotoxic effect, manifested by deformation and discoloration of the buckwheat stems. MCPA as well as mixture containing metazachlor and clomazone significantly increased the content of neutral detergent fibre (NDF), its fractions (hemicellulose, cellulose and lignin) and proteins compared with the control treatment. However, they caused a significant decrease in the ash content. Compared to the untreated control buckwheat seeds, application of MCPA caused an increase in the content of the following compounds: neutral detergent fibre (1.2%), hemicellulose (0.8%), cellulose (11.1%), lignin (0.6%) and protein (2.03%); mixture metazachlor and clomazone increase: neutral detergent fibre (0.8%), hemicellulose (0.5%), cellulose (11.3%), lignin (0.43%) and protein (0.60%). Each of the surveyed phytotoxic substances caused stress to buckwheat plants, with MCPA applied in the stage of two true leaves at a dose of 750 g ha^{-1} being the most aggressive stressor that caused deformation of the buckwheat plants, decreased yield (0.68 t ha^{-1}) and increased protein content (2.03%) in the buckwheat grains.

Key words: active substance, clomazone, *Fagopyrum esculentum*, metazachlor, 2-methyl-4-chlorophenoxyacetic acid (MCPA), phytotoxicity.

Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) is an important human food in some areas of the world. It is consumed in many Asian countries, including Japan, Korea and China, and is a popular and valued food source, especially in central and eastern European countries. Furthermore, buckwheat is utilised in medicine and industrial production. The protein content of buckwheat grains grown in the central European climatic zone ranges from 8.5% to 19% (Christa, Soral-Śmietana, 2008). The most remarkable feature of buckwheat grains is the lack of gluten, which makes it suitable as a food source in the diets of coeliac sufferers. Buckwheat proteins are rich in lysine – an amino acid that is lacking in other cereals, thus limiting the biological value of other cereal proteins (Christa, Soral-Śmietana, 2008; Vojtíšková et al., 2012; Lu et al., 2013). Starch is the major component of buckwheat grains, and its content varies from 59% to 70% of the dry mass. Moreover, buckwheat grains contain from 5% to 11% of fibre. The soluble fraction ranges from 3% to 7%, while the insoluble fraction is from 2% to 4% (Dziedzic et al., 2012; Vojtíšková et al., 2012; Górecka et al., 2014; Wang et al., 2017). Dietary fibre has a positive physiological effect on the gastrointestinal tract and also significantly influences the metabolism of other nutrients (Giménez-Bastida et al., 2015). Buckwheat grains contain from 1.5% to 4% of total lipids, with the content of raw fat in buckwheat flour exceeding 3%. Free lipids isolated from buckwheat grains constitute 2.5% of dry matter, whereas bound lipids are about 1.3% DM. Furthermore, buckwheat seeds contain

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2–2.5% of minerals and vitamins of an antioxidative nature, such as vitamin E and trace quantities of β-carotene (Hęś et al., 2012). Buckwheat grains and hulls consist of a number of components with pro-health properties and biological activity, i.e. flavonoids and flavones, phenolic acids, condensed tannins, phytosterols and fagopyrins (Vojtíšková et al., 2012; Dziedzic et al., 2015; Klepacka, 2015; Živkas et al., 2016). Buckwheat groats contain free phenolics (4.5–17.1 mg of gallic acid (GA) equivalent g^{-1}).

Increasing focus on the beneficial effects of buckwheat for human health, such as lowering blood pressure, cholesterol metabolism and having a low glycaemic index, has resulted in attention being currently paid to buckwheat products as a functional food (Lu et al., 2013). The quantities of these nutrients in seeds depend on different factors, for example, the cultivars, environmental factors and stress (Qin et al., 2010; Lu et al., 2013; Sakalauskaitė et al., 2013; Sobhani et al., 2014; Podolska, 2016; Dębski et al., 2018). Buckwheat, just like other plants, is subjected to different types of stress, namely biotic and abiotic. Chemical crop protection against pests significantly reduces the occurrence of pathogens, but, at the same time, may have an adverse effect on the life processes of the plants, resulting in morphological changes, decreased seed viability or cytological abnormalities. Plant protection agents cause the reduction of chlorophyll content in the plant as well as chloroplast degeneration. The results obtained by Dębski et al. (2018) indicated that glyphosate, when, compared with a control treatment, does not affect all flavonoids in the cotyledons of common buckwheat seedlings to the same extent. The level of the C-glycosides of luteolin and apigenin was reduced by glyphosate to a lesser extent than the level of o-glycosides of quercetin. Metazachlor blocks protein synthesis and cell division in plants. Its uptake occurs via the roots and hypocotyls of plants. Clomazone blocks the formation of carotenoids and is absorbed mainly by the roots, and to a lesser extent, through the leaves. Application of clomazone caused chlorosis (tissue bleaching). MCPA works similarly to indole-3-acetic acid (IAA), disrupting the hormonal balance of plants. It is absorbed by the leaves and roots (a selective and systemic herbicide – it moves within the plant) (Korbas et al., 2017).

Disturbances in the functioning of the basic physiological responses of crops may cause adverse changes in their photosynthetic apparatus, which in turn, leads to the disruption of photosynthesis and transpiration, and consequently to decreased yield. Plants, defending themselves against the adverse effects of stress factors, produce a defence mechanism, the effect of which is the changes in the production of chemical compounds (Podolska, Dietrych-Szostak, 2010; Kumar, 2012; Podolska, 2016; Dębski et al., 2018). Under drought stress, plants synthesise a set of new proteins or change their fractional and amino acid composition (Al-Tawaha et al., 2017). Podolska (2016) has shown that the amounts of albumins and globulins are not significantly affected by soil moisture; however, soil moisture had a significant effect on prolamin content in buckwheat seeds. The drought stress at a level of 30% soil water capacity from the flowering stage to harvest time resulted in a decrease in prolamin content, mainly of the gliadin content, compared with optimal soil moisture (60% of soil water capacity). Kwiatkowski (2010) suggests that buckwheat grains accumulate higher amounts of fat in dry years than in the wet years.

The content of antioxidant compounds, including flavonoids, is strongly determined by abiotic stress during the growing period. UV radiation stimulates the activity of enzymes in the phenylpropanoid pathway, and there is some evidence that it influences the rutin content in plants. A comparison of the different treatments revealed that the highest amounts of rutin were in plants grown under ambient radiation, followed by plants cultivated under enhanced UV-B and then under reduced UV-B radiation (Kreft et al., 2002). Rutin accumulation in buckwheat is also associated with the response to drought and cold stress. A higher rutin concentration in buckwheat grains is associated with water deficit at the level of 30% of soil water capacity from the period of flowering to seed setting compared with optimal soil moisture of 60% of soil water capacity (Kwiatkowski, 2010).

Buckwheat is very sensitive to pesticides. In fact, most studies have shown that herbicides are effective in reducing the number of common weeds in buckwheat crops, but certain active substances used in these products evoke stress reactions in buckwheat, manifested by growth inhibition, chlorosis, and the decrease in plant mass or plant height (Dębski et al., 2018). Such stress-induced changes may affect the quality and composition of the final product used for food production. Therefore, it is very important to determine the effect of active substances on the chemical composition of buckwheat seeds and the level of pesticide residues.

The purpose of the research was to assess the influence of the active phytotoxic substances present in commonly used herbicides; on the morphology of the plants and the chemical composition (neutral detergent fibre, lipids, protein and ash content) of buckwheat seeds. This is, to our knowledge, the first study investigating the stress-induced effect of a metazachlor and clomazone mixture and 2-methyl-4-chlorophenoxyacetic acid (MCPA) on buckwheat.

Materials and methods

Plant material. A field experiment was conducted in 2013–2015 at the Experimental Station in Osiny (51°35′, 21°55′), Institute of Soil Science and Plant Cultivation – State Research Institute, Pulawy, Poland. The experiment was conducted following a randomised complete block design with three replications. Sowing density was set at 2.8 million seeds per hectare. The area of harvested plots was 15 m^2. The common buckwheat (*Fagopyrum esculentum* Moench) cultivar 'Kora' was sown on a pseudopodsolic soil (spring barley was the fore-crop), with extractable phosphorus (P) 9.54 mg kg^{-1}, exchangeable potassium (K) 12.0 mg kg^{-1} and acidity (pH_{KCl}) 6.4. Plots were fertilised with 60 kg ha^{-1} N, 60 kg ha^{-1} P and 60 kg ha^{-1} K. Three active substances of herbicides: metazachlor (750 g ha^{-1}), clomazone (96 g ha^{-1}) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) (750 g ha^{-1}), were used in this experiment. Application of the metazachlor and clomazone mixture took place after sowing, whereas, that of MCPA was at the stage of two true leaves. The control was plots not using the herbicides.

The weather conditions in all experimental years were favourable for the growth of buckwheat. There was no frost after sowing. The temperature in July favoured the flowering of buckwheat (Table 1).

Table 1. Precipitation and temperature at the experimental site for the growing period (2013–2015)

Month	Precipitation mm			Temperature °C		
	2013	2014	2015	2013	2014	2015
May	104	189	114	15.2	14.3	13.5
June	116.4	121	33	18.2	16.8	17.9
July	49.8	63	54	19.3	20.9	20.4
August	11.1	17.5	4.6	19.3	18.3	22.6

Evaluation of the phytotoxic effect of the studied active substances on the plant development was visually estimated seven days after herbicide application at the flowering stage and at the start of the seed formation stage with an assessment based on OEPP/EPPO (Malinowski, 2006), using a scale of 1 to 9, where 1 means no crop injury and 9 means complete crop destroyed.

Methods. The dry matter content (DM) was determined according to AOAC 2001.12:2001 (Determination of water/dry matter (moisture) in animal feed, grains and food (plant tissue)).

The content of neutral detergent fibre (NDF), consisting of cellulose, hemicellulose and acid detergent fibre (ADF), was determined using the detergent method described by Van Soest (1963; 1967). Thermostable α-amylase was used to digest starch (McQueen, Nicholson, 1979). The reagents used to estimate the content of NDF were: neutral disodium versenate dehydrate, disodium tetraborate decahydrate, disodium hydrogen phosphate, ethylene glycol (pure) (Poch, Poland) and redistilled water. The reagents used to estimate the content of ADF were sulfuric acid 1 N (pure for analysis) (Poch, Poland) and N-cetyl-N,N,N-trimethylammoniumbromid (pure for analysis) (Merck, Germany). The reagent used to estimate the content of ADL was sulfuric acid 72% (pure for analysis) (Poch, Poland). Hemicellulose content was calculated from the difference between NDF and ADF. Cellulose content was calculated from the difference between ADF and ADL. The analyses were conducted using a Fibertec System M 1020 apparatus (Foss Tecator, Sweden).

%Hemicellulose = %NDF − %ADF, %cellulose = %ADF − %ADL.

The total content of lipids was determined by using petroleum ether; the solvent was evaporated using the gravimetric method (AOAC 996.01:2000 - Fat (total, saturated, unsaturated and monosaturated) in cereal products)). Soxtec HT6 equipment (Foss Tecator) was used for the extraction. The determination of lipids was done using the Soxhlet method. During this experiment, Soxhlet apparatus was used, which was the HT6 Foss Tecator extraction unit with petroleum ether.

Protein content was determined by the Kjeldahl method (AOAC 992.23:1992 - Crude protein in cereal grains and oils seeds). The conversion rate of 6.25 was used to convert nitrogen content to protein. The measurements were conducted using Kjeltec apparatus (Foss Tecator).

Ash content was assessed according to the determination method (AOAC 923.03:2005 - Ash of flour). Briefly, the sample to be analysed was placed in a porcelain crucible and incinerated in a muffle furnace at 550°C. The process lasted until all the organic substance was incinerated, after which the weight of the remained sample was measured.

Statistical analysis. The experiment was set up in a randomised block design in three replications. The results of the field and laboratory analyses were statistically processed using the software *Statistica*, version 7.1 (StatSoft Inc., USA). The influence of independent factors (variables) was analysed using the analysis of variance (*ANOVA*) method. The differences between mean values were compared using the Tukey's range test. Analysis of variance (multifactor *ANOVA*) was performed at a significance level of $\alpha = 0.05$, * – $\alpha < 0.05$, ns – not significantly different.

Results

The applied agents showed phytotoxicity to buckwheat crops (Table 2). The use of the metazachlor and clomazone mixture (prepared in the in the house of Institute of Soil Science and Plant Cultivation laboratory) caused leaf chlorosis and the reduction of plant height. These symptoms receded with the growth and development of buckwheat at the seed formation stage and disappeared completely prior to harvest. The use of MCPA caused an even more severe phytotoxic impact, which was manifested as deformed plants and discoloration of stems with anthocyan.

Table 2. Phytotoxicity of applied herbicides to buckwheat

Active substance	Buds showing stage		Seed fill stage	
	Phytotoxicity (1–9)	phytotoxicity symptoms	Phytotoxicity (1–9)	phytotoxicity symptoms
Metazachlor + clomazone	3	plants with symptoms of chlorosis of leaves	1	shorter plants, no symptoms of chlorosis of leaves
MCPA	6	slightly twisted plants with red stalk	5	slightly twisted plants with red stalk

Phytotoxicity (1–9) defined as: 1 – no symptoms of damage of buckwheat, 2 – very slight damage, 3 – slight damage, 4 – severe damage, not affecting yield levels, 5 – slight damage affecting yield levels, 6 – significant damage, 7 – strong damage, 8 – very strong damage, plant with very little chance of recovery, 9 – dead plants

Almost all the buckwheat traits analysed in this study were significantly affected by the active substances in the herbicides, but there was no interaction between year and experimental factor (active substances in the herbicides). Therefore, the results of the study are presented as an average of three years (Table 3, Figs 1–6).

The application of metazachlor and clomazone had no significant negative impact on the yield, leaf

Table 3. The influence of year and the active substances in the herbicides on the yield, yield components and chemical composition of buckwheat

Trait	Year (Y)	Active substances in the herbicides (H)	Interaction Y × H	CV
Yield	1.08	36.33*	0.22	11.0
Leaf area	1.71	204.36*	0.14	6.1
1000 seed weight	0.92	1.17	0.37	6.9
Plant height	2.16	138.75*	0.50	6.7
Number of inflorescences	5.31*	4.46*	3.26	22.8
Dry matter	1.86	1.53	1.12	0.2
Neutral detergent fibre (NDF)	6.87*	10.63*	1.65	1.5
Hemicellulose	3.06	576.7*	1.37	0.9
Cellulose	2.53	242.08*	0.77	0.7
Lipid	3.23	3.95	0.45	2.8
Lignin	3.89*	151.27*	0.34	0.5
Protein	4.11*	1069.77*	0.26	0.5
Ash	3.89*	151.27*	0.34	0.5

Results for one-factor analysis (*F*-test value with their significance) and the coefficient of variation (CV); * – significant at $p = 0.05$

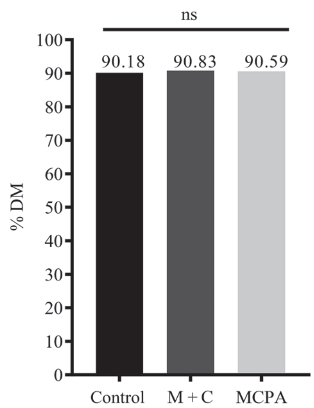
area, and number of inflorescences, but it caused a 31 cm reduction in plant height. MCPA significantly reduced buckwheat yield by 0.68 t ha^{-1} compared with the mechanical weed control treatment. MCPA caused the significant reduction of plant height (58.7 cm), leaf area (33%) and number of inflorescences per plant (6.6) (Table 4).

Table 4. Buckwheat yield and yield components (average from three year)

Trait	Active substance:		
	control	metazachlor + clomazone (M + C)	MCPA
Grain yield t ha^{-1}	1.24 a	1.18 a	0.56 b
Leaf area mm^2	2684 a	2731 a	891 b
1000 seed weight g	24.3 a	25.0 a	23.0 a
Plant height cm	94.7 a	63.0 b	36.0 c
Number of inflorescences	10.3 a	7.0 a	3.7 b

Note. LSD – least significant difference; means not sharing the same letter in each line are significantly different ($P < 0.05$), ns – not significant.

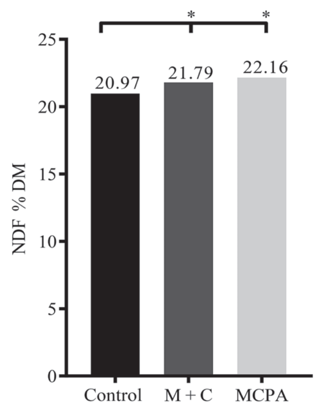
The influence of the substances on the chemical composition of buckwheat seeds is presented in Figures 1–6. The dry matter content was not significantly influenced by using herbicides compared with the control treatment (Fig. 1).



ns – not significant

Figure 1. Dry matter (DM) content in buckwheat seeds after application of herbicides metazachlor and clomazone (M + C) and MCPA

Figure 2 shows the content of neutral detergent fibre (NDF) in different samples during the experiment. The tested active substances caused a significant increase in NDF. There was a significant difference of 1.19 percentage points (pp) between the control and MCPA samples as well as between control and metazachlor and clomazone samples (0.82 pp). According to the NDF content, buckwheat seeds may be ranked as follows: control > metazachlor + clomazone > MCPA.

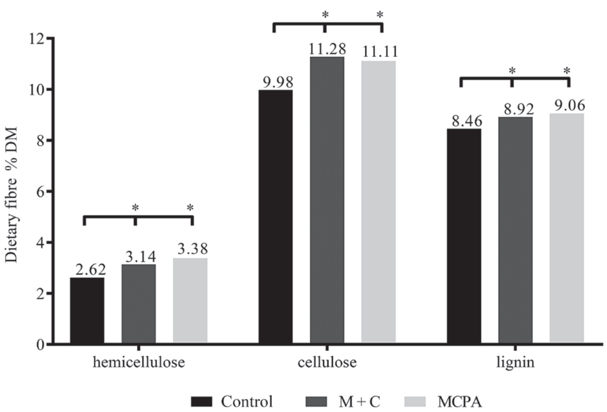


* – *P* value 0.01 < 0.05

Figure 2. Content of neutral detergent fibre (NDF) in buckwheat seeds after application of herbicides metazachlor and clomazone (M + C) and MCPA

Buckwheat seeds were characterised by a varied content of dietary fibre fractions (Fig. 3). The most abundant fraction in all samples, irrespective of the treatment, was the cellulose fraction followed by lignin. The average amount of hemicellulose equalled 3.05% DM, cellulose – 10.79% DM and lignin – 8.81% DM. The herbicides had a significant influence on the content of individual fibre fractions of buckwheat seeds. The lowest content of hemicellulose, cellulose and lignin was found in the seeds of the control treatment. The use of

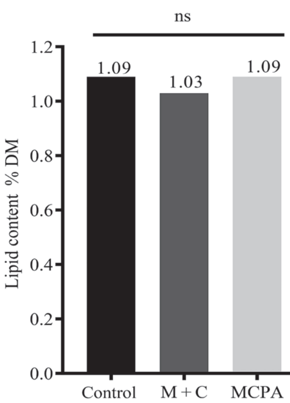
MCPA resulted in an increase of hemicellulose, cellulose and lignin by 0.76, 1.13 and 0.6 pp, respectively, in comparison with the control. A smaller increase in hemicellulose and lignin content (0.52 and 0.46 pp, respectively) when treating with the metazachlor and clomazone mixture was detected; however, a 1.3 pp. higher content of cellulose fractions was recorded.



Note. Harvest of the buckwheat seeds took place at the end of August; * – *P* value 0.01 < 0.05.

Figure 3. Content of the fractions of dietary fibre (hemicellulose, cellulose and lignin) in buckwheat seeds after application of herbicides metazachlor and clomazone (M + C) and MCPA

There was no significant influence of herbicides on the lipid content in buckwheat seeds (Fig. 4).

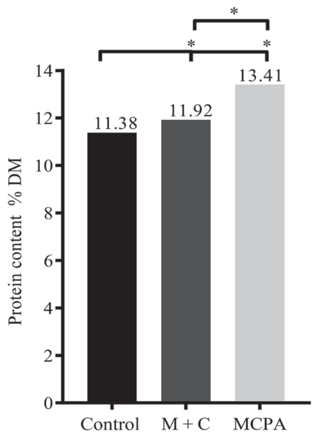


Note. Harvest of the buckwheat seeds took place at the end of August; ns – not significant.

Figure 4. Lipid content in buckwheat seeds after application of herbicides metazachlor and clomazone (M + C) and MCPA

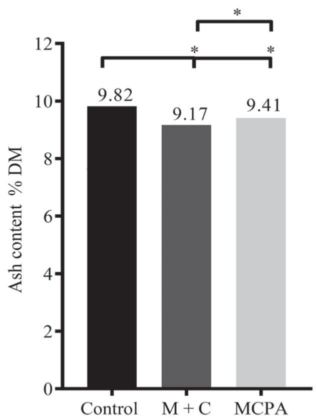
All the samples contained high levels of protein averaging 12.24% DM (Fig. 5). There was a significant difference between the control and the other investigated samples. Application of MCPA increased the amount of protein by 2.03 pp compared with the control treatment, while metazachlor and clomazone caused slight changes in the protein content of buckwheat seeds (by 0.54 pp).

The average ash content of the samples was 9.47% DM (Fig. 6). Application of MCPA and the metazachlor and clomazone mixture caused a slight reduction in the amount of ash in the buckwheat seeds by 0.41 and 0.54 pp, respectively, compared to the control treatment.



* – *P* value 0.01 < 0.05

Figure 5. Protein content in buckwheat seeds after application of herbicides metazachlor and clomazone (M + C) and MCPA



* – *P* value 0.01 < 0.05

Figure 6. Ash content in buckwheat seeds after application of herbicides metazachlor and clomazone (M + C) and MCPA

Discussion

The currently used plant protection products, which are a group of compounds of biological activity, not only get rid of unwanted weeds, but also can affect metabolism, leading to changes in the chemical composition of various crop seeds (Mollers, Albrecht, 1994; Murawa, Warmiński, 2005; Tekalign, Hammes, 2012; Pacanoski et al., 2014; Urban et al., 2014; Dębski et al., 2018). This study focused on the impact of three active substances of herbicides: metazachlor, MCPA and clomazone on the yield level and chemical composition of buckwheat seeds. To our knowledge, this was the first such study conducted in common buckwheat (*Fagopyrum esculentum* Moench). The active substances of the herbicides applied during the growth of buckwheat exhibited varied modes of action. Metazachlor blocks protein synthesis, clomazone blocks the formation of carotenoids, and MCPA acts similarly to auxin – indolilo-3-acetic acid (IAA), disrupting the hormonal balance of the plants (Gołębiowska, Badowski, 2015). These substances cause plant stress reactions. Stressors in plants can take different forms, affect the levels of yields and plant mass or cause changes in the plant structure and in the size of leaves (Szeleźniak et al., 2010; Jovonovic et al., 2011). They may also affect the hormonal balance, the efficiency of the photosynthetic apparatus, which, in turn, may cause permanent or intermittent changes of the size of biomass, the colouring of the leaves, etc. The plants, defending themselves against a stressor, produce larger amounts of organic compounds or enzymes (Podolska, Dietrych-Szostak, 2010; Rakszegi et al., 2014; Al-Tawaha et al, 2017).

MCPA turned out to be a more severe stressor for buckwheat plants. It caused deformities in plant structure and reduced the level and characteristics of yield structure, such as the height of plants or the number of inflorescences. Metazachlor and clomazone did not adversely affect the yield levels in thousand grain weight (TGW), leaf surface or the number of inflorescences. They only limited the height of plants and, immediately after application, caused the chlorosis of leaves, which then receded together with the growth and development of plants. However, symptoms suggest that MCPA is a stronger stressor for buckwheat than metazachlor or clomazone. Previous studies indicate that herbicides applied at the stage of true leaves exhibit different phytotoxicity to buckwheat. Szeleźniak et al. (2010) proved that clopyralid applied at a dose of 60 g ha^{-1} is not harmful to buckwheat plants, while MCPA applied at a dose of 750 g ha^{-1}, rimsulfuron at 125 g ha^{-1} and linuron at 675 g ha^{-1} caused shortening of buckwheat plants, while flurochloridone at 69 g ha^{-1} caused shorter plants and curly leaves with yellow spots. At the same time, these studies have proved that, despite the phytotoxic effect on buckwheat plants, the yield in the herbicide-applied plots was significantly higher than that in the mechanical weed control plots. Research by Pacanoski et al. (2014) indicates that metazachlor applied post sowing at a dose of 1056 g ha^{-1} was effective in destroying weeds and had a significant influence on carrot yield increase and quality.

The application of the above mentioned active substances on the chemical composition of buckwheat grains resulted in quantitative changes of certain chemical compounds. Research on the impact of these substances on the chemical composition of plants other than buckwheat was the subject of previous studies. However, our study is the first to investigate the influence of MCPA, metazachlor and clomazone on the condition of buckwheat plants and some of the nutrients or functional substances.

Murawa et al. (2001) proved that the application of metazachlor after sowing at a dose of 1200 g ha^{-1} in mustard contributed to the increase in the protein content by 1.7% as well as the reduction of the fat content. The increase in the protein content was at the same level in all the tested cultivars, whereas the fat reduction depended on the cultivar; in 'Borowska' it was 3.4%, while in 'Nakielska' – 2.4%.

Research on metazachlor was also conducted by Murawa and Warmiński (2005). Their studies on oilseed rape have shown that the use of deltamethrin (Decis) alone increases the fat content in the seeds by two percentage points compared to the control, whereas the additional use of metazachlor and quinmerac (Butisan) causes only a 1.03 percentage points increase in the fat content. These results suggest that metazachlor did not affect the lipid content in the seeds.

Gołębiowska and Badowski (2015) studied the impact of a mixture of metazachlor with clomazone, metazachlor with chinomerac and clomazone alone on the morphology and yields of winter oilseed rape. The following soil herbicides were applied in the experiments: clomazone at a dose of 120 g ha^{-1}, metazachlor and clomazone at doses of 750 and 99.9 g ha^{-1} as well as metazachlor and chinomerac at doses of 999 and 249 g ha^{-1} in the form of mixtures. They found that the symptoms of phytotoxic impact of the tested mixtures were less severe than those of clomazone alone. Compared to clomazone, all the other substances mentioned above did not influence the content of protein or fat in the seeds.

In this study, the application of metazachlor with clomazone caused an increase in the protein content by 0.54%, whereas the lipid content remained unaffected in buckwheat seeds. In contrast, a decrease of lipid content was noted by Mollers and Albrech (1994) when metazachlor was applied. Enzymes, such as C18:2 desaturase as well as the eicosenic-CoA elongase are assumed to be preferentially inhibited by these herbicides due to an exposed position in the chloroplast envelope and the membranes of the endoplasmic reticulum, respectively. Metazachlor mixed with clomazone caused an increase in the content of NDF (0.8%), hemicellulose (0.5%), cellulose (11.3%), lignin (0.4%) and protein (0.6%), but it did not affect the content of dry matter.

A beneficial impact of MCPA on the content of the NDF (1.2%), hemicellulose (0.8%), cellulose (11.1%), lignin (0.6%) and protein (2.03%) was provided. However, there was no impact on the lipid content and dry mass. Application of MCPA caused a reduction in the amount of ash in the buckwheat seeds. A 2.03% increase in protein content was founded, which was very high and is important to note. A beneficial impact of MCPA on the protein content has been confirmed in other studies. Urban et al. (2014) proved a significant impact of MCPA (300 g ha^{-1}) and Dicamba (40 g ha^{-1}) applied at the tillering stage in oats on the increase in the protein content of about 0.6%. They did not record, however, the impact of MCPA and Dicamba on the content of starch, the total pentozans, soluble pentozans or ash. Similarly, Noworolnik (2009) has shown a beneficial impact of MCPA (300 g ha^{-1}) and Dicamba (40 g ha^{-1}) applied at the tillering stage on the protein content and quality. It increased more valuable fractions, such as the albumins and globulins in the protein, while reducing the amount of less-valuable prolamines. The research of Klimont (2007) on winter wheat confirmed that the application of MCPA and Dicamba (Chwastox D) at a dose of 805 g ha^{-1} of MCPA and 89 g ha^{-1} of Dicamba at the tillering stage causes a significant increase in the protein content compared to the control treatment.

The increase in the protein content, however, did not exceed one percent. He also recorded an increase in the amount of gluten. Compared to the control, the increase of gluten content was 3.15%. Klimont (2007) has recorded a beneficial influence of MCPA and Dicamba (Chwastox D) on the protein content in the grains of winter wheat, whereas they did not prove a significant impact on the protein content in the grains of spring wheat, barley or triticale. The increase of the protein content was significant and amounted to 0.6%. Kwiatkowski et al. (2017) applied MCPA at 360 g ha^{-1} at the beginning of the tillering stage and noticed a significantly higher fibre content in millet grains compared to the control treatment (without herbicide). The difference in fibre content was 1.3 pp. They also found no significant influence of the application of MCPA in millet on the protein content, amino-acid content, o-dihydroxy phenol and selenium, but proved that 2.4-D + fluroxypyr and tribenuron-methyl + fluroxypyr increased the essential amino acid index (EAAI) from 56.7 to 71.5 and 69.8, respectively. They also found that under the influence of the herbicides discussed, the grains had a higher content of certain amino acids, such as glutamine, proline, cysteic acid and valine. The grains also showed a higher accumulation of valuable essential amino acids (lysine, methionine and tryptophan) compared to the other weed control treatments. There was no significant effect of the tested substances on the content of fibre and starch in the seeds of different cultivars of spring barley and millet (Klimont, 2007). In contrast, the Petri dishes laboratory experiment conducted by Kumar (2012) with different concentrations (0, 50,100, 200, 400, 800 and 1200 ppm) of herbicides showed that the application of 2,4-dichlorophenoxy acetic acid and isoproturon decreased the protein content and carbohydrate content in winter wheat cultivars. They also observed a reduction of the band intensity on electrophoretic protein band analysis due to herbicide use.

The tested active substances of MCPA and metazachlor with clomazone have a significant effect on buckwheat seed chemical composition, including NDF, hemicellulose, cellulose, lignin and protein. Identification of specific abiotic stresses that affect grain composition in ways that have implications for food quality needs more investigation.

Conclusions

1. Application of the metazachlor and clomazone mixture caused the chlorosis of buckwheat leaves. These symptoms were transitional and faded away prior to harvest. The use of this mixture caused a reduction in the yield of buckwheat.

2. MCPA (2-methyl-4-chlorophenoxyacetic acid) showed a strong phytotoxic impact, involving plant deformities and stem discoloration. The application of MCPA resulted in a reduction of plant height, leaf area, number of inflorescences per plant, and a considerable yield decrease.

3. The MCPA-metazachlor-clomazone mixture increased the contents of neutral detergent fibre (NDF) and its fractions and protein, but reduced the content of ash in the seeds of buckwheat cultivar 'Kora'. However, they had no impact on the lipid content in the dry mass. The analyses of the impact of the tested substances on phytotoxicity, yield and yield structure characteristics and chemical composition of buckwheat seeds showed that MCPA is a stronger stressor for buckwheat than metazachlor and clomazone. The most valuable active substances would ideally enhance the nutritional value of buckwheat, but would not negatively affect the growth and yield of buckwheat.

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Abiotinio streso įtaka grikių cheminei sudėčiai ir derliui

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Santrauka

Vertingų maisto medžiagų kaupimasis grūduose priklauso nuo daugelio veiksnių, taip pat ir biotinio bei abiotinio streso ir herbicidų poveikio. Tyrimo tikslas – nustatyti metazachloro bei klomazono mišinio ir 2-metil-4-chlorfenoksiacto rūgšties (MCPA) įtaką sėjamojo grikio (*Fagopyrum esculentum* Moench) sėklų neutraliais tirpalais išplautos ląstelienos, lipidų, baltymų ir pelenų cheminei sudėčiai. Lauko eksperimentas atliktas 2013–2015 metais. Nustatyta, kad kitą dieną po grikių sėjos panaudotas 750 g ha^{-1} metazachloro ir 96 g ha^{-1} klomazono mišinys buvo toksiškas ir išryškėjo lapų chlorozė. Jos simptomai išsilaikė iki žydėjimo tarpsnio pabaigos ir palaipsniui išnyko sėklų formavimosi tarpsniu. Dviejų tikrųjų lapelių tarpsniu išpurkšta 750 g ha^{-1} MCPA norma buvo labiau fitotoksiška ir lėmė grikių stiebų deformaciją bei spalvos pakitimą. MCPA, kaip ir metazachloro bei klomazono mišinys, grikių sėklose esmingai padidino neutraliais tirpalais išplautos ląstelienos, jos frakcijų (hemiceliuliozės, celiuliozės bei lignino) ir baltymų kiekį, palyginus su kontroliniu variantu. Tačiau šios cheminės medžiagos esmingai sumažino pelenų kiekį. Palyginus su kontroliniu variantu, grikių sėklose MCPA 1,2 % padidino neutraliais tirpalais išplautos ląstelienos, 0,8 % hemiceliuliozės, 11,1 % celiuliozės, 0,6 % lignino ir 2,03 % baltymų kiekį; metazachloro ir klomazono mišinys 0,8 % padidino neutraliais tirpalais išplautos ląstelienos, 0,5 % hemiceliuliozės, 11,3 % celiuliozės, 0,43 % lignino ir 0,60 % baltymų kiekį.
Abi tirtos fitotoksinės medžiagos grikių augalams sukėlė stresą, o 750 g ha^{-1} MCPA normos panaudojimas dviejų tikrųjų lapelių tarpsniu buvo agresyviausias stresorius, sukėlęs grikių augalų deformaciją, derliaus sumažėjimą (0,68 t ha^{-1}) ir padidinęs baltymų kiekį (2,03%) grikių grūduose.

Reikšminiai žodžiai: *Fagopyrum esculentum*, fitoksiškumas, klomazonas, metazachloras, 2-metil-4-chlorfenoksiacto rūgštis (MCPA), veiklioji medžiaga.