Journal of Elementology ISSN 1644-2296

Głowacka A, Klikocka H., Onuch J. 2015. *Content of zinc and iron in common bean seeds (Phaseolus vulgaris L.) in different weed control methods.* J. Elem., 20(2): 293-303. DOI: 10.5601/jelem.2014.19.2.499

CONTENT OF ZINC AND IRON IN COMMON BEAN SEEDS (*PHASEOLUS VULGARIS* L.) IN DIFFERENT WEED CONTROL METHODS

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Abstract

A field experiment was conducted in 2010-2012 on a private farm located in the village of Frankamionka in the administrative district (*powiat*) of Zamość, on brown soil with slightly acidic pH, and the average abundance of zinc and iron. The experiment was set up in a random split-plot design with four replications, with seven methods for controlling weed infestation: 1) no weeding control, 2) hand weeding control, 3) linuron (Afalon dyspersyjny 450 SC), 4) linuron + metribuzin (Afalon dyspersyjny 450 SC + Mistral 70 WG), 5) linuron + chlomazone (Harrier 295 ZC), 6) linuron + chlomazone + metribuzin (Harrier 295 ZC + Mistral 70 WG), 7) bentazon (Basagran 480 SL twice). The objective of the study was to determine the effect of weeding control methods on the content of iron and zinc in the seeds of cv. Jaś Karłowy common bean *(Phaseolus vulgaris* L.).

The highest seed yield, on average 29.39 dt ha^{-1}, was obtained on the plots where weeds were controlled by the application of the herbicides Harrier 295 ZC + Mistral 20 WG just after sowing. The lowest seed yield was harvested on unweeded plots – 6.77 dt ha^{-1} on average.

Statistical analysis showed a significant effect of the weed control methods and the weather conditions in growing seasons on the content of iron and zinc in bean seeds. The lowest amount of zinc, an average of 36.11 mg kg^{-1}, was found in beans from unweeded plots. The use of the herbicides Afalon dyspersyjny 450 SC + Mistral 70 WG, Harrier 295 ZC and Harrier 295 ZC + Mistral 70 WG significantly increased the zinc content compared to the un weeded control and manual weeding.

The highest iron concentration, on average 75.12 mg kg^{-1}, was observed in seeds from unweeded plots. Significantly less iron accumulated in beans from plots weeded manually and by application of the herbicides Harrier 295 ZC and Harrier 295 ZC + Mistral 70 WG.

Key words: herbicides, hormesis, micronutrients, bentazon, linuron, chlomazone, metribuzin.

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INTRODUCTION

Leguminous plant seeds are a valuable source of plant protein, group B vitamins, cellulose, complex carbohydrates and fiber (Firatligil-Durmuş et al. 2010). According to Kahlon et al. (2005), low morbidity from cardiovascular diseases in Asia is the result of high leguminous seed consumption (110 g daily per capita) there. In Western Europe, consumption of seeds of leguminous plants reach 3.5 kg per capita per year. In Poland, the consumption of legumes was 5 kg per capita in the 1980s, but has drastically decreased since then, down to about 1.5 kg of seeds per capita (Podleśny 2005).

Common bean (*Phaseolus vulgaris* L.) is an important world grain legume and vegetable. Among all the major food legumes, the common bean is the world's third most important bean after soybeans (*Glycine max* (L.) Merr.) and peanut (*Arachis hypogea* L.) (Lin et al. 2008). It plays a significant role in human nutrition, being an economically viable source of protein, dietary fiber, minerals and vitamins for people in developing and developed nations (Graham, Ranalli 1997, Santalla et al. 2001). Also, the content of iron and group B vitamins, such as thiamine and riboflavin, in these seeds is rather high (Iqbal et al. 2006). In Poland, the common bean cultivation for dry seeds has recently covered over 20 thousand hectares and it is the main leguminous plant cultivated for consumption (Głowacka 2010, Łabuda 2010).

Iron and zinc are two of the micronutrients, i.e. essential elements needed in small amounts for proper human nutrition. Both of these minerals are crucial to human well-being and an adequate supply of iron and zinc helps to prevent iron deficiency anaemia and zinc deficiency, two prevalent health concerns of the developing world (Blair et al. 2009). According to the World Health Organization (WHO), iron and Vitamin A deficiencies are the most common forms of malnutrition, leading to severe public health consequences (Carvalho et al. 2012). The content and uptake of micronutrients by plants depend on many factors, e.g. plant species and variety, plant development phases, soil and climatic conditions, and agrotechnical procedures (Moraghan et al. 2002, Korus et al. 2005, Bowszys et al. 2009, Gugała, Zarzecka 2010). Weed control is one of the most important elements of the dry bean cultivation technology because weeds can drastically reduce the yield and quality of bean seeds (Hekmat et al. 2008). Dry bean producers in Poland have a limited number of herbicides for weed control, for example bentazon, S-metolachlor and propachizafop. Thus, research is needed to identify herbicides that provide consistent control of weeds in dry bean with an adequate margin of crop safety. Studies conducted in other countries indicate the possibility of using of active substances, such as linuron or chlomazone in weed control in common bean (Soltani et al. 2006, Sikkema et al. 2008). In addition, farmers in south-eastern Poland sometimes use herbicides, such as Linurex 500 SC, Harrier 295 ZC or Mistral 70 WG for weed control in common bean, although they are not registered for use in this crop. According to Devine and Hall (1990) and Geiger and Bestman (1990), herbicides used for weed control may affect the uptake of nutrients by modifying the root respiration, transpiration, the permeability of cell membranes and ion antagonism. Thus, the aim of the study was to assess the impact of different weed control methods on the seed yield and on the content of iron and zinc in common bean dry seeds.

MATERIAL AND METHODS

A field experiment was conducted in 2010-2012 on a private farm located in the village of Frankamionka in the administrative district (*powiat*) of Zamość (50°73'N 23°65'E). The experimental field was located on brown soil, slightly acidic (pH in 1 mol KCl 6.8), with 20 g kg^{-1} of organic matter and an average content of available forms of zinc and iron (7.2 mg Zn kg^{-1}; 899 mg Fe kg^{-1}). The experiment was set up in a random split-plot design with four replications. The subject of the research was the cultivar Jaś Karłowy of common bean *(Phaseolus vulgaris* L.) .

The following weed control methods were examined:

1) no weeding control;
2) hand weeding control;
3) linuron 450 g ha^{-1} (Afalon dyspersyjny 450 SC 1.0 dm^3 ha^{-1});
4) linuron 675 g ha^{-1} + metribuzin 14 g ha^{-1} (Afalon dyspersyjny 450 SC 1.5 dm^3 ha^{-1} + Mistral 70 WG 20 g ha^{-1});
5) linuron 375 g ha^{-1} + chlomazone 67.5 g ha^{-1} (Harrier 295 ZC 1.5 dm^3 ha^{-1});
6) linuron 375 g ha^{-1} + chlomazone 67.5 g ha^{-1} + metribuzin 14 g ha^{-1} (Harrier 295 ZC 1.5 dm^3 ha^{-1} + Mistral 70 WG 20 g ha^{-1});
7) bentazon 600 g ha^{-1} + bentazon 600 g ha^{-1} (Basagran 480 SL 1.25 dm^3 ha^{-1} + Basagran 480 SL 1.25 dm^3 ha^{-1}).

The herbicides Afalon dyspersyjny 450 SC, Harrier 295 ZC and Mistral 70 WG were applied just after common been sowing (to 3 hours). Basagran 480 SL was applied twice: first 3 week after bean sowing, and next ten days letter. Then, to avoid the impact of weeds that were not completely destroyed or germinated at a time when the herbicides stopped working, weeds were removed manually, as needed.

Common bean was grown on a site where the previous crop was winter wheat. Mineral fertilization was applied uniformly in the amounts of N – 30, P – 32 and K – 79 kg ha^{-1} (ammonium nitrate, triple superphosphate, potassium sulphate). All fertilizers were applied once before sowing. Tilling was carried out using the traditional method in accordance with the agrotechnical recommendations for this plant. In subsequent years of the research, beans were sown on 29th and 30$^{th\ April}$ and on 2th May. The distance between rows was 45 cm and the density was 35,000 plants per hectare. Before sowing, the bean seeds were mixed with Vitavax 200 FS (a.i. carboxin 200 g l^{-1} + thiram 200 g l^{-1}). Beans were grown for dry seeds and harvested by hand on 28th August and on 3nd and 6th September, depending on the year of research. After harvesting, seed yields were determined. Then, the zinc and iron content was determined by atomic adsorption spectrophotometry (AAS) after wet mineralization in analytically pure HNO_3 according to PN-EN ISO 6869:2002. Based on the content of the elements in common bean seeds and the yield volume, the uptake of zinc and iron was calculated. The results were analyzed statistically by analysis of variance using Statistica PL. Differences between the means were assessed by the Tukey's test, with 95% probability.

The weather conditions varied over the years of the study. Rainfall was the highest in 2010, when it exceeded the long-term average. Heavy precipitation was recorded in May, July and August (Figure 1). Precipitation in the second (2011) and third (2012) growing seasons was on a similar level. The average monthly temperatures in each year were higher than the long-term average. Particularly warm were the years 2010 and 2012, when the sum of temperatures from April to September was from 2981 to 2989°C, while the long-term average equalled 2690°C (Figure 1).

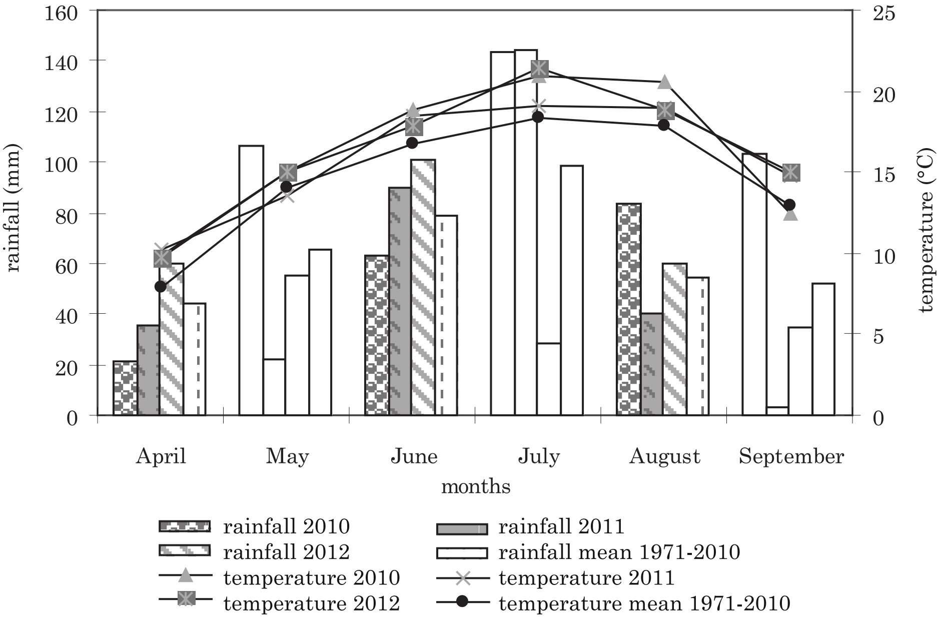

Fig. 1. Rainfall and air temperature in the months April-September as compared to the long-term means (1971-2010), according to the Meteorological Station in Zamość

RESULTS AND DISCUSSION

The analysis of the results showed that the method of weed control and the course of the weather in different growing seasons had a significant effect on seed yields of common bean. Bean seed yields in Poland amount to about 26.0 dt ha^{-1}. In the present study, the highest yield at 29.39 dt ha^{-1} was achieved in 2012, which was characterized by uniform distribution of precipitation (Table 1). The lowest yield of common bean seeds was recorded in the 2011 growing season (22.69 dt ha^{-1}), which had a marked deficiency of rainfall in May. This could adversely affect the germination and initial growth of common bean. In addition, excessive rainfall observed in July could have reduced the pod formation. As expected, the smallest yield was obtained for the unweeded control. HEMSS (1985) reports that concurrent weeds throughout the beans' growing season can reduce bean yields by up to 60%. Among the weed control methods tested, the highest yield, 30.99 dt ha^{-1}, was obtained by using the herbicides linuron + chlomazone+ metribuzin (Harrier 295 ZC + Mistral 20 WG), and it was significantly higher than that from the control weeded by hand or other herbicide combinations. An idea that poisons in small doses can be stimulating was suggested over 100 years ago. According to APPLEBY (1998), hormesis is a common phenomenon in many plants that occurs with a wide range of herbicides. However, using herbicides to stimulate crops and increase yields, other than by reducing competition from weeds, is not easy. CEDERGREEN et al. (2007) reported that the physiological and molecular mechanisms behind the growth hormesis in plants are not well investigated. Plants have hormones, and it is possible that some of the hormetic responses stem from the activation of a plant's hormonal systems. Among the combinations assessed, the use of linuron (Afalon dyspersyjny 450 SC) and of the post-emergence herbicide bentazon

Table 1

Yield of common bean seeds (dt ha^{-1})

Weed control	Years			Mean value
	2010	2011	2012	
No weeding control	6.87^{a}	5.64^{a}	7.81^{a}	6.77^{a}
Hand weeding control	27.94^{d}	25.7^{cd}	32.90^{cd}	28.85^{d}
Afalon dyspersyjny 450 SC	26.91^{bc}	24.06^{b}	32.29^{bc}	27.74^{bc}
Afalon dyspersyjny 450 SC + Mistral 70 WG	27.38^{cd}	25.14^{c}	33.61^{d}	28.71^{d}
Harrier 295 ZC	26.84^{bc}	26.0^{d}	32.98^{cd}	28.63^{cd}
Harrier 295 ZC + Mistral 70 WG	30.26^{e}	28.15^{e}	34.58^{e}	30.99^{e}
Basagran 480 SL + Basagran 480 SL	25.9^{bc}	24.06^{b}	31.57^{b}	27.17^{b}
Mean	24.59^{b}	22.69^{a}	29.39^{c}	-
$LSD_{(0.05)}$ between: years (II) – 0.86; weed control methods (I) – 0.93; Interaction I × II – n.s. weed control methods in years: 2010 – 1.04, 2011 – 0.97, 2012 – 1.13				

(Basagran 480 SL) influenced the yield of beans in the least favourable way (Table 1). The variance analysis confirmed no significant interaction between the years of research and applied weed control methods.

Iron deficiency is the primary cause of nutritional anaemia and the most common nutritional deficiency in the world. Iron absorption can be improved by increasing dietary factors that enhance iron absorption, by decreasing factors that inhibit iron absorption, or by increasing the iron content of the diet by fortification or supplementation. Zinc deficiency is associated with poor growth, loss of appetite, skin lesions, impaired taste acuity and impaired immune response (Whitthaker 19998). The common bean (*Phaseolus vulgaris* L.) is an important source of protein and minerals. It supplies all of the iron that humans require for metabolism and provides 25% of the daily requirements of magnesium and copper as well as 15% of potassium and zinc (Nchimbi-Msolla, Tryphone 2010). The course of weather conditions in growing seasons significantly affected the zinc content in the seeds of common beans. The smallest amount of these nutrient was found in 2011, the year characterized by low rainfall in the months of May and August. Very small precipitation in May, compared with the other years of research, could slow down the germination and initial growth of plants. The higher zinc content was recorded in 2010 (Table 2). The influence of precipitation on the release of nutrients to the soil solution and on the absorption of micronutrients and their content in plants is also confirmed by Stępniak-Sołyga and Wojtasik (2003) Klikocka (2011) and Głowacka (2013).

Meyer et al. (2013) reported the zinc content of bean seeds at the level of 21-25 mg kg^{-1}. In the study by BLAIR et al. (2009), the zinc content in seeds of different varieties of beans varied within a wide range from 17.7 mg kg^{-1} to 42.4 mg kg^{-1}. In the present study, the zinc content of bean seeds ranged from 32.42 mg kg^{-1} to 65.32 mg kg^{-1}, and therefore can be regarded as high.

Table 2

Content of zinc in common bean seeds (mg kg^{-1} d.m.)

Weed control	Years			Mean value
	2010	2011	2012	
No weeding control	41.20^{a}	32.42^{a}	34.73^{a}	36.11^{a}
Hand weeding control	56.31^{b}	41.38^{b}	48.87^{b}	48.85^{b}
Afalon dyspersyjny 450 SC	54.79^{b}	41.91^{b}	49,46^{b}	48.34^{b}
Afalon dyspersyjny 450 SC + Mistral 70 WG	65.32^{d}	51.89^{d}	60.44^{d}	59.21^{d}
Harrier 295 ZC	63.89^{c}	46.33^{c}	56.17^{c}	55.46^{c}
Harrier 295 ZC + Mistral 70 WG	62.74^{c}	46.85^{c}	57.52^{c}	55.70^{c}
Basagran 480 SL + Basagran 480 SL	54.91^{b}	42.01^{b}	47.68^{b}	48.20^{b}
Mean	57.02^{b}	43.20^{a}	50.71^{c}	-
$LSD_{(0.05)}$ between: years (II) – 2.71; weed control methods (I) – 3.34; Interaction I × II – n.s. weed control methods in years: 2010 – 1.43, 2011 – 2.97, 2012 – 2.06				

The lowest amount of zinc, on average of 36.11 mg kg^{-1}, was found in beans with no weeding control. The analysis of variance confirmed a significant effect of the weed control methods on zinc accumulation in bean seeds (Table 2). The application of the following herbicides: linuron + metribuzin (Afalon dyspersyjny 480 SC + Mistral 70 WG), linuron + chlomazone (Harrier 295 ZC) and linuron + chlomazone + metribuzin (Harrier 295 ZC + Mistral 70 WG) significantly increased the zinc content compared to the unweeded control and manual weeding. Differences in the zinc content between variant with linuron (Afalon dyspersyjny 480 SC), bentazon + bentazon (Basagran 480 SL + Basagran 480 SL), and the hand weeding control were not statistically significant. Similarly, Gugała and Zarzecka (2010) observed a significant effect of herbicides on the content of magnesium and calcium in pea seeds. However, Adomas and Piotrowicz-Cieślak (2007) found no significant effect of herbicides on the content of nutrients in the seeds of blue lupine and yellow lupine.

The common bean was confirmed to be an excellent source of iron for human consumption (Carvalho et al. 2012). The iron concentration of bean seeds is affected by the plant's genotype and the environment (Beebe et al. 2000, Frossard et al. 2000). According to the literature, the iron content in bean seeds ranges from 40.0 mg kg^{-1} to 84.0 mg kg^{-1} (Blair et al. 2009, Meyer et al. 2013). In our study, the content of iron in bean seeds ranged from 50.89 mg kg^{-1} to 98.43 mg kg^{-1}. The highest iron content was recorded in the growing season 2010 (78.40 mg kg^{-1} on average). The lowest iron content (58.91 mg kg^{-1}) was recorded in 2011, the year with unevenly distributed rainfall (Table 3).

The largest content of iron (75.12 mg kg^{-1} on average) was found in beans without weeding (Table 3). Beans weeded manually and by application of the herbicides: linuron + chlomazone (Harrier 295 ZC) and linuron + chlomazone + metribuzin (Harrier 295 ZC + Mistral 70 WG) accumulated si-

Table 3

Content of iron in common bean seeds (mg kg^{-1} d.m.)

Weed control	Years			Mean value
	2010	2011	2012	
No weeding control	90.43^{e}	55.87^{ab}	79.06^{e}	75.12^{e}
Hand weeding control	77.51^{c}	62.13^{d}	71.04^{c}	70.22^{d}
Afalon dyspersyjny 450 SC	75.26^{b}	54.89^{a}	68.87^{b}	66.34^{b}
Afalon dyspersyjny 450 SC + Mistral 70 WG	78.40^{c}	56.83^{ab}	71.90^{c}	69.04^{c}
Harrier 295 ZC	76.31^{bc}	68.47^{e}	69.08^{b}	71.28^{d}
Harrier 295 ZC + Mistral 70 WG	80.85^{d}	56.61^{b}	74.03^{d}	70.49^{cd}
Basagran 480 SL + Basagran 480 SL	69.81^{a}	57.23^{bc}	63.32^{a}	63.45^{a}
Mean	78.40^{c}	58.91^{a}	71.00^{b}	-
$LSD_{(0.05)}$ between: years (II) – 1.02; weed control methods (I) – 1.07; Interaction I × II – n.s weed control methods in years: 2010 – 2,16, 2011 –1.73, 2012 – 2.03				

gnificantly less of these micronutrient. The use of the following combinations of herbicides: linuron (Afalon dyspersyjny 480 SC) and bentazon + bentazon (Basagran 480 SL + Basagran 480 SL) was the least favourable to the accumulation of iron by common beans. According to CAKMAK (2004), high-yielding crops may contain smaller amount of nutrients than crops that produce less biomass because they are "diluted" in the biomass of the plant. This phenomenon was confirmed by KLIKOCKA and GŁOWACKA (2013) with reference to micronutrient concentrations in potato tubers.

The smallest uptake of zinc and iron with the seeds was found on the control plots without weeding (Table 4). The highest uptake of zinc was observed after the application of linuron + metribuzin (Afalon dyspersyjny 450 SC + Mistral 70 WG) and linuron + chlomazone + metribuzin (Harrier 295 ZC + Mistral 70 WG), while the highest iron uptake was recorded in plots with the application of linuron + chlomazone (Harier 295 ZC) and linuron + metribuzin (Afalon dyspersyjny 450 SC + Mistral 70 WG). Regarding zinc, this effect was due to the seed yield and zinc content in the bean seeds, which is confirmed by the Pearson's correlation coefficients (Table 5). The uptake of iron was positively correlated with the seed yield, but there was a negative correlation between the uptake and content of iron in seeds. In the study by NCHIMBI-MSOLLA and TRYPHONE (2010), a positive correlation between

Table 4

Uptake of zinc and iron by common bean seeds (mean for 2010-2012)

Weed control	Uptake (g ha^{-1})	
	zinc	iron
No weeding control	24.40^{a}	56.27^{a}
Hand weeding control	140.9^{c}	188.2^{cd}
Afalon dyspersyjny 450 SC	134.1^{bc}	172.9^{b}
Afalon dyspersyjny 450 SC + Mistral 70 WG	170.0^{e}	213.9^{f}
Harrier 295 ZC	158.8^{d}	204.1^{ef}
Harrier 295 ZC + Mistral 70 WG	172.6^{e}	196.6^{de}
Basagran 480 SL + Basagran 480 SL	131.0^{b}	179.4^{bc}
$LSD_{(0.05)}$	7.3	14.8

Table 5

Correlation coefficients between yield and content and uptake of zinc and iron

Nutrients		Yield of seeds	Nutrients content	
			zinc	iron
Content	zinc	0.858***	-	-
	iron	- 0.811***	- 0.427*	-
Uptake	zinc	0.972***	0.953***	-
	iron	0.971***	-	- 0.655**

the concentration of Fe and Zn in common bean seeds was observed. These results suggest that genetic factors for increasing iron and zinc are co-segregating with genetic factors for increasing zinc (Tryphone, Nchimbi-Msolla 2010). In our study, the iron content in common bean seeds was negatively correlated with the zinc content and with the seed yield (Table 5).

CONCLUSIONS

1. The highest yields of common bean seeds were obtained in the plots where weeds were controlled with linuron + chlomazone + metribuzin (Harrier 295 ZC + Mistral 20 WG) applied just after sowing.

2. Some of the herbicides examined in the study significantly increased the zinc content compared with the unweeded control and hand weeding. The highest zinc content was associated with the application of three herbicides: linuron + metribuzin (Afalon dyspersyjny 450 SC + Mistral 70 WG); linuron + chlomazone (Harrier 295 ZC) and linuron + chlomazone + metrobuzin (Harrier 295 ZC + Mistral 70 WG).

3. The highest iron content was obtained when there was the unweeded control. The smallest iron content was associated with the application of linuron (Afalon dyspersyjny 450 SC) and bentazon + bentazon (Basagran 480 SL + Basagran 480 SL).

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