РЕАКЦИЯ НА СЛЪНЧОГЛЕДОВИ ХИБРИДИ CLEARFIELD КЪМ ПРЕПОРЪЧИТЕЛНИ И ПО-ВИСОКИ ДОЗИ НА ХЕРБИЦИДА *ИМАЗАМОКС*

RESPONSE OF SUNFLOWER CLEARFIELD HYBRIDS TO BOTH RECOMMENDABLE AND HIGHER DOSES OF THE *IMAZAMOX* HERBICIDE

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Abstract

Sunflower is an important oilseed crop worldwide and its successful cultivation requires careful control of weed species. The Clearfield® technology in sunflower has been developed to allow the use of imidazolinone herbicides as a post-emergence weed control option. The Clearfield hybrids are tolerant to *imazamox*, aherbicide of the imidazolinone group with the trade name *Pulsar 40*, produced by the BASF Chemical Company. However, after *imazamox* treatment a temporary yellowing of the meristem tissue of the sunflower leaves is observed. The herbicidal stress increases in combination withun suitable weather conditions such as long term high temperatures and drought.

The aim of the study was to examine the response of Clearfield sunflower hybrids to higher doses of the *imazamox* herbicide. Five hybrids were examined: *LG 56.58, Tektonic, Alego, Meldimi* and*Primis*. The doses of *imazamox* treatment were as follows: non-treated control,the recommendable dose of *imazamox* (4.8 g a.i./da= 120 ml /da *Pulsar 40*) and doubled *imazamox* doses (9.6 g a.i. / da = 240 ml/da *Pulsar 40*). Treatment with the herbicide was done at 4^{th}-6^{th}-leave stage. The biometric and photosynthetic analyzes were carried out after the treatment. The photosynthetic leaf gas exchange, quantum yield of PSII photochemistry and growth parameters (height, fresh weight and leaf area) were determined.

The obtained results showed that the treatment with the recommendable dose of *imazamox* caused a temporary negative impact on the photosynthetic performance and growth of the sunflower plants, which recovered after 14 days.The doubled dose of *imazamox* seriously damaged the existing top leaves of the sunflower plants, but the newly developed leaves were without toxicity symptoms. The studied sunflower cultivars expressed good tolerance to *imazamox*, applied in the recommendable dose.

Key words: Clearfield, growth response, *imazamox*, photosynthesis, sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus*) is one of the most important oilseed crops worldwide and sunflower oil is commonly used because of its health benefits and good taste. Broad leaf weeds are known to cause considerable yield losses in sunflower production (Blamey et al., 1997) competing with it for moisture and nutrients. Sunflower is a good competitor with weeds for light in later growth stage, but does not cover the ground early enough to prevent weed establishment. Therefore, early season weed control is essential for good sunflower yield and annual weeds are on the primary focus of weed control. The most common method for weed management is herbicide treatment.

The Clearfield technology has been developed in sunflower to allow the use of imidazolinone herbicides as a post-emergence weed control option. The mode of action of imidazolinone herbicides is the inhibition of the enzyme acetohydroxy acid synthase (AHAS), the first common enzyme in the branched chain amino acid synthesizing pathway (Duggleby, 2000). Imidazolinone-tolerant sunflower hybrids (IMI-R) were developed by conventional breeding methods and have been commercialized as Clearfield® crops since 2003 (Pfenning et al., 2008). The Clearfield® production system consists of a combination of imidazolinone herbicides with imidazolinone resistant sunflower hybrids. While the conventional sunflower cultivars are imidazolinone sensitive, Clearfield® sunflower hybrids can survive an otherwise lethal application of these herbicides (Pfenning et al., 2008). Imazamoxis a selective herbicide of the imidazolinone group used against annual and perennial grasses and broadleaf weeds applied in a wide spectrum of crops (Hess et al., 2010). Imidazolinone herbicides are widely used because they have a broad spectrum of weed control activity, low usage rates, and low mammalian toxicities (Shaner et al., 1996).

However, imazamox application may cause injury in imidazolinone resistant crops such as wheat (Geier et al., 2004; Hanson et al., 2006) and also temporary yellowing and reduction of plant height in IMI-R sunflower hybrids (Pfenning et al., 2008). These effects can be more pronounced when crops are growing under stressful environmental conditions (heat, drought, waterlogged soils, etc.) (Pfenning et al. 2008), which occur more frequently in recent years. One of the most typical symptoms of damage due to imidazolinone treatment is leaf chlorosis (Ochagavia et al., 2014) caused by reduction of chlorophyll ***a*** content (Netherland et al., 2009), which inevitably leads to disruption of the photosynthetic apparatus.

Photosynthesis is an integral physiological process, which is very sensitive to different stresses, including herbicides. The evaluation of photosynthetic performance is presently recognized as a suitable approach for detection of herbicide-induced plant disorders. During the last years, several *in vivo* measured photosynthetic analyses, such as leaf gas exchange and chlorophyll fluorescence, have become widely used in ecophysiological studies due to their sensitivity and rapidity.

Different Clearfield sunflower hybrids are currently commercially available. They differ in their expression of different alleles of the same locus (*Ahasl1* locus) in the AHAS1 gene, conferring resistanceto AHAS inhibiting herbicides, which result in the different tolerance levels to the herbicides of the imidazolinone group (Sala et al., 2012). The evaluation of imidazolinone resistance in plants is based mainly on *in vivo* AHAS activity measurements (Reinbolt et al., 2005) or different plant growth parameters (Breccia et al., 2011). Surprisingly, photosynthetic parameters have not been often used for evaluation of photosynthetic performance of imazamox-treated plants. The aim of this study was to evaluate both growth and photosynthetic responses of five Clearfield sunflower hybrids to the recommended dose and double dose of the herbicide imazamox.

MATERIALS AND METHODS

Plant material and experimental design. The five commercial Clearfield sunflower hybrids CL 56.58, Tektonik, Alego, Mildimi and Primis were randomly selected for tolerance examination to the herbicide imazamox.

Pot-soil experiments were carried out in the greenhouse of the Department of Plant Physiology and Biochemistry in 2011. The sunflower plants were grown in pots filled with 5 kg dry soil taken from the experimental field of Agricultural University of Plovdiv. When plants reached 4-6 leafstage, two-factorial experimental deign was set up: first factor – genotype (the 5 hybrids given above) and second factor – herbicide imazamox treatment (non-treated control, treated with the recommendable field imazamox dose (4.8 g a.i./da= 120 ml Pulsar 40/da) and the double imazamox dose (9.6 g a.i./da = 240 ml/da Pulsar 40). Each of the variants was replicated in three pots, 4 plants per pot. During the vegetation, watering was carried out through the drainage up to 60-70% soil moisture.

Analyses. Photosynthetic response was monitored by both leaf gas exchange and chlorophyll fluorescence analyses carried out on the7th and 14th days after treatment (DAT). Net photosynthetic rate (A) was determined by a portable photosynthetic system LCA-4 (ADC, Hoddesdon, England) in the 3rd pair leaves between 10:00 and 12:00 at suitable environmental conditions (light intensities between 800 and 1000 μmol m^{-2} s^{-1} and temperature 20 and 25 ^oC). Quantum yield of PSII photochemistry (Genty et al., 1989) was measured with a portable pulse amplitude modulated photosynthesis yield analyzer (Mini-Pam, Heinz Walz, Effeltrich, Germany) equipped with a standard 2030-B leaf clip holder in the same leaves and environmental conditions.

On the14thDAT the plants were harvested and the following biometrical parameters were measured: height (cm), leaf area (cm^2) and fresh weight of plants (g).

Statistical analysis was performed using one way ANOVA (for $P<0.05$). Based on ANOVA results, a Duncan test for mean comparison was performed, for a 95% confidence level, to test for significant differences among treatments of each cultivar. In the figures, different letters (a, b, c) express significant differences.

RESULTS AND DISCUSSION

Data presented in Table 1 shows that the treatment of sunflower plants with imazamox in the recommendable dose (4.8 g/da) resulted in a significant decrease of A ($P < 0.05$), with one exception in cv. Mildimi. The inhibition of A varied between 33 and 41%. The decrease of A was higher in plants treated by the double imazamox dose, as expected. Our results confirm the opinion (Pfenning et al., 2008; Ochagavia et al., 2014) that the imazamox can cause a slight negative impact on tolerant plants even when it is applied in the recommendable dose. The results obtained in our study correspond also with those of Gaston and co-workers which show an inhibition of Ain imazethapyr treated pea plants (Gaston et al., 2002). Our results provide evidence that the sunflower hybrid Mildimi showe better tolerance to imazamox treatment compared to the other cultivars. The net photosynthetic rate in imazamox-treated plants from this cultivar was almost not affected by both the recommendable and the doubled imazamox dose.

Net photosynthetic rate represents the state of the overall photosynthetic process – CO_2 assimilation. In fact, the photosynthetic process involves reactions at different functional levels – pigment level, primary light reactions, thylakoid electron transport reactions, dark-enzyme stroma reactions as well as slow regulatory feedback processes. The herbicide treatment can influence directly or indirectly any of these processes. Chlorophyll fluorescence is another possible approach for indirect judgment of plant photosynthetic performance. This arises from the fact that fluorescence is complementary to the alternative pathways of utilization of absorbed sunlight energy, which are photochemistry (photosynthesis) and heat dissipation. Briefly, the fluorescence yield is the highest when the photochemistry and heat dissipation is the lowest.

The results presented in Table 1 show also that Y of herbicide-treated sunflower plants were diminishedin the studied cultivars, but to different degrees – from 9 to 26% in the recommendable dose and similarly in the doubled one. The tendency was significant in cvs. Tektonik and Alegro treated with the recommended dose and cvs. Tektonik and Primis – in the higherimazamox dose. It shouldbe mentioned also that the decrease of Y was obviously smaller than that of A, which provide sevidence that the primary light reactions are less affected than other photosynthetic processes. The results obtained in our study correspond with the findings of Sousa et al. (2013) that the imidazolinone herbicides can cause inhibition of photosynthetic electron transport reactions.

On the 14th DAT (Table 2) the values of A in both non treated (control) and treated with the recommendable imazamox dose (4.8 g a.i./da) plants were similar, indicating significant recovery of the photosynthetic apparatus. The Y values showed the same tendency – the determined values were insignificantly different from the control plants. Photosynthetic measurements of plants treated with the double imazamox dose were not performedon the 14th DAT due to visual damages of the respective leaves, such as necrotic spots or existing of both yellowing and epinasty symptoms. Nevertheless, it is necessary to point out that the developing new leaves were without toxicity symptoms.

Table 1. Net photosynthetic rate (A) and quantum yield of PSII photochemistry (Y) of plants from different Clearfield sunflower hybrids 7 days after treatment with the herbicide imazamox

Cultivars/ Treatments	Control	4,8 g/da imazamox	9,6 g/da imazamox
	A (μmol m^{-2} s^{-1}) (In parenthesis -% from control)		
LG 56.58	20,48 (100) a	11,99 (59) c	14,96 (73) b
Tektonik	19,20 (100) a	12,11 (63) b	10,40 (54) b
Alego	22,41 (100) a	14,10 (63) b	10,67 (47) b
Mildimi	20,98 (100) a	20,39 (97) a	19,91 (95) a
Primis	18,68 (100) a	12,57 (67) b	9,49 (51) b
	Y (In parenthesis -% from control)		
LG 56.58	0,192 (100) a	0,172 (90) a	0,167 (87) a
Tektonik	0,254 (100) a	0,188 (74) b	0,194 (76) b
Alego	0,259 (100) a	0,219 (79) ab	0,194 (78) b
Mildimi	0,264 (100) a	0,239 (91) a	0,228 (86) a
Primis	0,213 (100) a	0,187 (88) a	0,137 (65) b

The values represent the mean of three biological replicates. Different letters (a, b, c) express significant differences (P < 0.05) with in the treatments of each cultivar.

Table 2. Net photosynthetic rate (A) and quantum yield of PSII photochemistry (Y) of plants from different Clearfield sunflower hybrids 14 days after treatment by the herbicide imazamox

Cultivars/ Treatments	Control	4,8 g/da imazamox
	A (µmol m^{-2} s^{-1}) (In parenthesis -% from control)	
LG 56.58	17,82 (100) a	17,05(96) a
Tektonik	14,80 (100) a	14,66(99) a
Alego	15,99 (100) a	16,10 (101) a
Mildimi	16,91 (100) a	17,81(105) a
Primis	17,22 (100) a	13,41(78) b
	Y (In parenthesis -% from control)	
LG 56.58	0,218 (100) a	0,192 (88) a
Tektonik	0,281 (100) a	0,223 (79) a
Alego	0,216 (100) a	0,226 (104) a
Mildimi	0,239 (100) a	0,230 (96) a
Primis	0,207(100) a	0,202 (98) a

The values represent the mean of three biological replicates.
Different letters (a, b, c) express significant differences (P < 0.05) within the treatments of each cultivar.

The biometrical analyses of plants, carried out on the 14thDAT, are presented in Figure 1. The results show that the growth of imazamox-treated plants from all sunflower Clearfield hybrids was inhibited, but to a different extentin-between cultivars.

The height of plants from cvs. LG56.58, Tektonik and Meldimi (Figure 1 B), treated with the recommendable imazamox dose, was significantly reduced than no treated (control) plants, while the plants from cvs. Alego and Primis were only slightly (not significantly) lower. The height of plants treated with the double imazamox dose was strongly diminished in all tested cultivars.

The treatment of sunflower plants with imazamox resulted in inhibition of fresh weight accumulation (Figure 1 A). The fresh weight of plants treated with the recommendable dose imazamox was significantly lower than the respective controls at cvs. LG56.58 and Alego and tended to decrease inother cultivars. Again, the double dose imazamox strongly inhibited the plants from all cultivars.

The leaf area formation in imazamox treated plants was also influenced (Figure 1 C). The herbicide, applied inthe double dose, caused inhibition (about 50% or higher)of this parameter, while inthe recommendable dose – slight but significant inhibition only in plants from cvs. LG56.58 and Primis.

The obtained results in our study are in a good correspondence with other studies which show decreased growth of sunflower plants treated with the double dose of imazamox, 7th DAT (Bozic et al., 2012). The recommendable dose of the herbicide also led to growth reduction, but the plants were less inhibited and had better recoverability.

CONCLUSIONS

In general, considering our results obtained from photosynthetic and biometric measurements of Clearfield sunflower hybrids treated with recommendable and double imazamox doses, we can point out the following conclusions:

1. The treatment with the herbicide imazamox causes an inhibition of growth and photosynthetic performance in IMI-R Clearfield sunflower hybrids. The inhibition is lesspronouncedinthe plantstreated with the recommendabledose (4.8 g a.i./da= 120 ml/da Pulsar 40) and significantly higher in the plants treated with the exceeded imazamox dose.

2. The sunflower plants from all tested cultivars treated with the recommendable imazamox dose recovered their photosynthetic performance on the 14th DAT, while those with the doubled dose did not express such vitality.

3. The biometrical parameters of sunflower plants treated with the recommendable dose of imazamox on the 14th DAT were lower as compared with the no-treated controls, but the growth of newly developed leaves was not retarded.

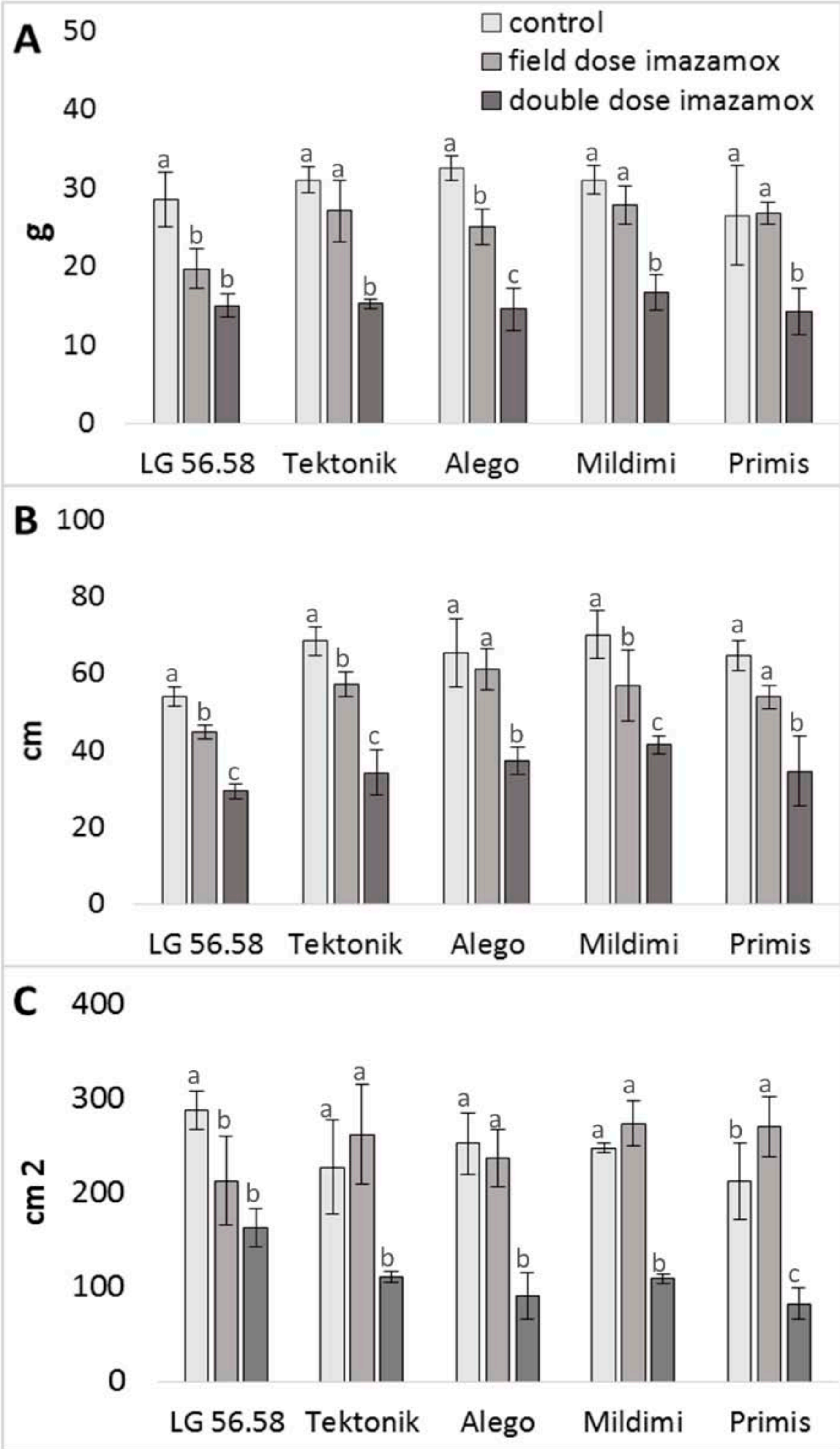

Fig. 1. *Biometrical parameters: A – weight of plants; B – height of plants and C – leaf area, of Clearfield sunflower hybrids treated with field dose (4.8 g a. i./da) and double dose (9.6 g a. i. /da) of herbicide imazamox, 14 days after treatment. The values represent the mean of three biological replicates. Different letters (a, b, c) express significant differences (P < 0.05).*

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