Original scientific paper
Journal of Central European Agriculture, 2023, 24(1), p.126-136

DOI: /10.5513/JCEA01/24.1.3668

Photosynthetic performance and productivity of maize (*Zea mays* L.), exposed to simulated drift of imazamox and subsequent therapy application with protein hydrolysates

---

Фотосинтетична активност и продуктивност на царевица (*Zea mays* L.), изложена на симулиран дрифт от хербицида имазамокс и лечебно приложение на протеинови хидролизати

Dobrinka BALABANOVA[1] (✉), Nesho NESHEV[2], Mariyan YANEV[2], Lyubka KOLEVA-VALKOVA[1], Andon VASSILEV[1]

[1] Agricultural University of Plovdiv, Department of Plant Physiology, Biochemistry and Genetics, Plovdiv 4000, 12 Mendeleev str, Bulgaria

[2] Agricultural University of Plovdiv, Department of Agriculture and Herbology, Plovdiv 4000, 12 Mendeleev, Bulgaria

✉ Corresponding author: dobrinka_balabanova@abv.bg

Received: June 27, 2022; accepted: November 3, 2022

ABSTRACT

The use of herbicides is a traditional method for weed control in crop-producing systems. Along with the high effective weed control, herbicides might cause phytotoxicity for crop plants, due to insufficient herbicide selectivity, combining herbicide treatment with unsuitable meteorological conditions, long-term persistence of herbicide in the soil or off-target transfer of the herbicide – drift. Imazamox is a selective herbicide of imidazolinone group, used to control annual and perennial weeds in imidazolinone-resistant (IMI-R) crops. Protein hydrolysates (PHs) are a group of plant biostimulants containing small peptides and free amino acids, reported to ameliorate plant abiotic stress tolerance, including herbicide phytotoxicity. This report evaluates the damaging effect of simulated imazamox drift on growth, photosynthetic performance and productivity of maize plants as well as the efficiency of foliar application by protein hydrolysates as therapy means. The received results demonstrated that the simulated imazamox herbicide drift has a strong inhibiting effect on maize plants. This is well illustrated by the retarded growth of maize plants, their disrupted photosynthetic activity and productivity losses. The foliar supply of PHs to imazamox damaged maize plants ameliorates their photosynthetic performance, growth and crop productivity.

Keywords: plant biostimulants, protein hydrolysates, imazamox, herbicide drift, maize, photosynthesis

РЕЗЮМЕ

Използването на хербициди е традиционен метод за контрол на плевелната растителност при полски култури. Наред с високоефективния контрол на плевелите, хербицидите могат да причинят фитотоксичност при културните растения поради недостатъчна селективност, третиране при неподходящи метеорологични условия, остатъчни количества в почвата или при отлитане хербицида извън целевата култура – хербициден дрифт. Имазамоксът е селективен хербицид от групата на имидазолиноните, прилаган за контрол на едногодишни и многогодишни плевели в комбинация с устойчиви хибриди (IMI-R). Протеиновите хидролизати (PHs) са група растителни биостимуланти, съдържащи малки пептиди и/или свободни аминокиселини, за които е известно, че подобряват толерантността на растенията към абиотичен стрес, включително хербицидна фитотоксичност. Настоящото проучване има за цел да установи ефекта на симулиран дрифт на хербицида имазамокс върху растежа, фотосинтетичната активност и продуктивността на царевични растения, както и да оцени ефективността на листното приложени биостимуланти от групата на протеиновите хидролизати като терапевтично средство. Получените резултати показват, че симулираният хербициден дрифт оказва силен инхибиращ ефект върху царевичните растения. Това се вижда ясно от потиснатия растеж на царевичните растения, нарушената им фотосинтетичната активност и загубата на продуктивност. Листното прилагане на протеинови хидролизати върху увредените от имазамокс царевични растения подобрява фотосинтетичната им активност, растежа и продуктивността на културата.

Ключови думи: растителни биостимуланти, протеинови хидролизати, имазамокс, хербициден дрифт, царевица, фотосинтеза

INTRODUCTION

The use of herbicides is a traditional method of weed control in crop technologies. Although modern herbicides have increasingly selective properties, in some cases, they can provoke toxic effects on agricultural plants (herbicide phytotoxicity) (Cobb and Reade, 2010). Such cases include (1) insufficient herbicide selectivity (de Carvalho et al., 2009); (2) combining herbicide treatment with unsuitable meteorological conditions (Jursík et al., 2020); (3) long-term persistence of herbicide in the soil (Chowdhury et al., 2020); (4) off-target transfer of the herbicide - drift (Vieira et al., 2020) and others.

The herbicide drift can be characterized as either primary or secondary movement (Bish et al., 2021). Primary movement is an off-target drift of the herbicide at the time of application, while secondary movement occurs after the herbicide application. For example, vapor drift is a form of secondary movement due to herbicide volatilizing into the atmosphere. Both types of herbicide drift can induce chronic or acute toxicity on non-target crops.

Chronic phytotoxicity is visualized as growth retardation, the appearance of chlorosis, necrosis, various deformation symptoms and often leads to loss of productivity. The harmful effect depends on the dose of the assimilated herbicide, crop sensitivity, environmental conditions and others. However, significant yield reduction without visible phytotoxicity symptoms has also been observed (Janaki et al., 2017).

Imazamox is a selective herbicide of imidazolinone group, used to control annual and perennial weeds in imidazolinone-resistant (IMI-R) crops (Pfenning et al., 2008). Its mechanism of action is inhibition of acetohydrohy acid synthase (AHAS), a key enzyme in the biosynthetic pathway of the branched-chain amino acids. The treatment of susceptible plants with an imidazolinone herbicide leads to growth inhibition, yellowing the growing point (Shaner, 1991) and others. Despite high imazamox selectivity, in some cases, it may provoke temporary phytotoxicity even in IMI-R crops (Balabanova et al., 2020).

The negative effects of imazamox drift have been observed in the agronomic practice but rarely were the subject of research experiments. Webster et al. (2016) reported significant growth inhibition and yield losses caused by imazamox drift on rice. Deeds et al. (2006) found that imazamox drift can produce injury even in the lowest dose (1/100 of the dose used for other crops) and nearly complete kill and yield loss of wheat at the highest (1/3 of the dose), regardless of plant growth stage at application. Information concerning crops sensitivity to imazamox is scarce and for maize is fully missing. Meanwhile, the information about maize sensitivity to imazamox is essential from a practical viewpoint, as in Bulgaria this crop is often sown in a neighbourhood with IMI-R-grown sunflower.

The plant biostimulants (PBs) are a new generation of products available on the agricultural market. EU Regulation classifies them as fertilizing products, "the function of which is to stimulate plant nutrition processes independent from the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (a) nutrient use efficiency, (b) tolerance to abiotic stress, (c) quality traits, or (d) availability of confined nutrients in the soil or rhizosphere" (Regulation (EU) No 2019/1009). PBs are biologically derived products containing small peptides, free amino acids, phytohormones, vitamins, organic acids, micro- and macronutrients, etc. They belong to several main groups such as protein hydrolysates, humic and fulvic acids, algae extracts, microbial products and others (du Jardin, 2015).

Protein hydrolysates (PHs) are a subgroup of plant biostimulants defined as mixtures of polypeptides and amino acids produced from protein sources using partial hydrolysis (Schaafsma, 2009). Their application has been reported to avoid or reduce production losses caused by unfavorable soil and environmental conditions, such as temperature, drought and salinity (Botta, 2013; Petrozza et al., 2014; Lucini et al., 2015). The database concerning the effects of PHs on the performance, growth and productivity of herbicide-damaged plants is very scarce.

Insufficient information about the effects of imazamox drift on maize plants motivated us to conduct a study aimed to: (1) establish chronic and lethal doses of imazamox treatment on young maize plants; (2) the long-term effect of imazamox-induced chronic phytotoxicity on both photosynthetic performance and yield of maize as well as (3) evaluate the efficiency of foliar application by protein hydrolysates as therapy means.

MATERIAL AND METHODS

Field experiments were conducted in the Experimental field of the Agricultural University of Plovdiv (Latitude 42°8'19'' N, Longitude 24°48'7" E, an elevation of about 202 m above mean sea level), in 2019 - 2020. The maize (*Zea mays* L.) hybrid P9241 (Pioneer Seeds Bulgaria) was grown in the experiments.

The soil of the Experimental field is classified as Mollic Fluvisols (Popova et al., 2012). The mechanical composition is average sandy-clay, not high humus content, and weak-alkaline reaction (pH_{H_2O} = 7.3). The content of available forms of nitrogen, phosphorus and potassium were as follows (mg/kg): NH_4 – 19.3; NO_3 – 16.6; P_2O_5 – 328; K_2O – 318. The soil nitrogen content was determined according to Kjeldahl method by distillation in apparatus of Parnas-Wagner (Tomov et al., 2009). Phosphorus and potassium content were measured colorimetrically (spectrophotometer Camspec M105) and photometrically (flame photometer PFP-7), respectively (Tomov et al., 2009).

Combined fertilization with 250 kg/ha with N:P:K (15:15:15) + 8% SO_3 + 0.01% Zn (Borealis - L.A.T Company, Austria) before sowing of the crop was done. The fertilizer was distributed to the whole experimental area with fertilizer spreader. In spring, dressing with 250 kg/ha NH_4 + NO_3 (34.4% Total N; Neochim PLC, Bulgaria) was performed. The fertilizer was introduced in the rows of maize with fertilizer applicator together with the first in-row tillage.

The harvest was done with Harvester "Wintersteiger", Germany.

The maize seeds were sown in April for both years. The climatic conditions during both experimental years were favorable for the germination, growth and development of maize plants. The average minimum and maximum monthly temperatures and precipitation during the vegetation period in 2020 are given in table 1.

Range-finding experiment

A small-scale field experiment with maize plants exposed to simulated drift by the herbicide imazamox (Pulsar Plus; BASF) was carried out in 2019. Two-factorial experiment with 12 variants was set up. The first factor was the time of imazamox treatment, presented by two plant growth stages, namely the 3 - 4 leaf and a 7 -8 leaf of maize plants. The second factor was the doses of foliar application by imazamox, adjusted to 5, 10, 20, 40 and 50 g a.i./ha. The treatments were done by backpack sprayer SOLO model 417 (Solo, Germany). The untreated (control) plants were sprayed with tap water. The volume of the applied solution was 250 L/ha. Each variant had three replications (plots) with a size of 5 m^2 and approximately 30 plants. The plants were harvested at 10th leaf stage and their growth parameters were measured.

Table 1. Average monthly precipitation, minimum and maximum monthly temperatures (°C) during the maize vegetation in 2020

	April	May	June	July	August	September
T min°	3.3	5.8	11.3	14.9	21.2	13.4
T max°	19.0	28.4	30.5	31.6	32.8	29.3
Precipitation (mm)	89.1	54.1	89.9	40.8	24.7	1.3

Field experiments

Two field experiments (experiment 2A and experiment 2B) were conducted in 2020.

The purpose of experiment 2A was to establish the effect of imazamox-induced chronic phytotoxicity on photosynthetic performance and maize grain yield. The experimental design included four variants, namely: (1) untreated by imazamox (control) plants; (2) plants treated by 5 g a.i. imazamox/ha; (3) and (4) plants, treated by 10 and 20 g a.i. imazamox/ha, respectively.

Experiment 2B aimed to establish whether or not the foliar application by protein hydrolysates would improve the photosynthetic performance and yield of imazamox-damaged maize. The experimental design included six variants, namely: (1) untreated by imazamox (control) plants; (2) plants treated by 10 g a.i. imazamox/ha; (3) plants, treated by 10 g a.i. imazamox/ha, followed by foliar application with Naturamin® WSP (0.5 g/ha); (4) plants, treated by 10 g a.i. imazamox/ha, respectively, followed by foliar application with Naturamin® Plus (2.5 L/ha); (5) plants, treated by 10 g a.i. imazamox/ha, respectively, followed by foliar application with Terra-sorb® (2.5 L/ha) and (6) plants, treated by 10 g a.i. imazamox/ha, respectively, followed by foliar application with Trainer® (2.5 L/ha). The applied protein hydrolisates are trade products of the following firms: Daymsa, Spain (Naturamin® WSP and Naturamin® Plus), Bioiberica, Spain (Terra-sorb®) and Italpollina, Italy (Trainer®).

Both trials were performed by the randomized block design in 3 replications with a plot size of 15 m^2. The herbicide treatment was applied at the 7-8th leaf of maize plants by a backpack sprayer. The biostimulant application in experiment 2 was made five days after the herbicidal treatment by the same sprayer. The volume of working solution in both experiments was 250 L/ha.

Studied parameters

Plant growth parameters

The height and fresh biomass of both roots and leaves of 10 randomly selected maize plants from each variant were measured 10 days after the herbicidal treatment (range-finding experiment).

Photosynthetic pigments

Leaf gas exchange and photosynthetic pigments content of the upper fully developed leaves were measured: (1) 5 days after the herbicide treatment in the experiment 2A and (2) 5 days after the biostimulant application in the experiment 2B.

Photosynthetic pigments (Chl.a, Chl.b and total carotenoids) in the leaves of maize plants grown in experiment 2A were extracted in 85% acetone, measured spectrophotometrically and calculated according to the formulae of Lichtenthaler (1987). The total chlorophyll content of maize plants grown in experiment 2B, was determined by chlorophyll meter CCM-300 (Opti-Sciences, USA).

Leaf gas exchange: The net photosynthetic rate on the fully developed upper leaves of maize plants grown in both experiments was determined between 10.00 and 12.00 o’clock with the open photosynthetic system LCpro+ (ADC, Hoddesdon, UK).

Yield: The grain yield of maize was determined at harvest. The maize grain yield was determined with a harvester for field plot trials “Wintersteiger”. The data from each plot was recalculated to establish the grain yields per hectare.

Statistical analysis: Statistical analysis was performed using analysis of variance (ANOVA). Based on ANOVA outcomes, a Dunkan's test for the main comparison at a 95% confidence level was applied. In the figures and tables, different letters (a, b, c) indicate significant differences between pairs of means.

RESULTS

Effects of increasing doses of imazamox on the growth of young maize plants (range-finding experiment)

The doses of 40 and 50 g/ha imazamox were highly toxic to the maize, the growth of plants stopped after the treatment and red color appeared on leaf blades. Due to the high toxicity, the treated plants died within 7-10 days after the treatment and therefore, here are presented results only of the lower imazamox doses (5, 10 and 20 g/ha).

The biometric parameters of the maize plants are shown on table 2. The results from the plants treated in the earlier (3 – 4) leaf stage show that the treatment of the susceptible maize plants with herbicide imazamox significantly inhibits their growth. This retardation is most pronounced on the variant with 20 g/ha imazamox dose with decreased plant height with 50% and root and above ground weight with 74% and 77%, respectively. The dose of 5 g/ha imazamox shows very weak and not significant growth suppression. The treatment of maize in 7-8 leaf phase also had a strong inhibitory effect on the plant vegetation. The plant height is inhibited by 20 g/ha imazamox with approximately 50% and root and above ground weight with 71% and 82%, respectively.

Effects of imazamox on photosynthetic performance and productivity of maize (experiment 2A)

The content of photosynthetic pigments and net photosynthetic rate are closely related physiological indicators commonly used to evaluate plant performance in normal and stress conditions (Sacramento et al., 2018). Our results show that the simulated off-target drifts with herbicide imazamox are causing an inhibitory effect on the photosynthetic pigment profile. The pigments content (Table 3) was significantly lower in imazamox treated plants than in untreated control.

Table 2. Growth parameters of maize plants, treated with different doses of imazamox. The values represent the mean of five biological replicates, ± SD. The significance levels of two way analysis of variance (ANOVA): * *P*=0.05, NS – not significance. LS – leaf stage, TR – treatment. In breckets is presented the percent of the untreated control

Variants	Height of plants (cm)	Weight (g)	
		Roots	Leaves
		Treatment at 3 – 4th leaf age stage	
Untreated (control)	93.0 ± 7.6 ^{a} (100)	21.9 ± 5.8 ^{a} (100)	101.5 ± 23.1 ^{a} (100)
5 g/ha imazamox	89.6 ± 8.7 ^{a} (96)	18.0 ± 4.9 ^{ab} (82)	94.2 ± 12.3 ^{a} (93)
10 g/ha imazamox	62.0 ± 8.2 ^{b} (66)	15.3 ± 3.1 ^{b} (70)	43.0 ± 9.5 ^{b} (42)
20 g/ha imazamox	46.2 ± 2.9 ^{c} (50)	5.7 ± 1.7 ^{c} (26)	23.6 ± 4.9 ^{b} (23)
		Treatment at 7 – 8th leaf age stage	
Untreated (control)	103.4 ± 15.7 ^{a} (100)	23.9 ± 2.6 ^{a} (100)	120 ± 14.3 ^{a} (100)
5 g/ha imazamox	98.6 ± 6.5 ^{a} (95)	18.1 ± 3.3 ^{b} (76)	90.6 ± 10 ^{a} (76)
10 g/ha imazamox	71.0 ± 6.5 ^{b} (67)	16.1 ± 2.3 ^{b} (67)	45.6 ± 5.5 ^{b} (38)
20 g/ha imazamox	51.4 ± 9.7 ^{c} (50)	6.9 ± 0.9 ^{c} (29)	21.2 ± 5.5 ^{c} (18)
Two Way ANOVA	LS - *	LS - NS	LS - NS
	TR - *	TR - *	TR - *
	LS x TR - NS	LS x TR - NS	LS x TR - NS

Values followed by different superscript letters (a, b, c) within columns differ significantly at *P*<0.05

Table 3. Photosynthetic pigments content [mg/g FW] in maize plants, treated with different doses of imazamox. The values represent the mean of five biological replicates, ± SD. In breckets is presented the percent of the untreated control

Variants	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	a/b	(a+b)/k
Untreated (control)	1.78 ± 0.11 ^{a} (100)	0.52 ± 0.02 ^{a} (100)	0.52 ± 0.02 ^{a} (100)	0.52 ± 0.02 ^{a} (100)	3.59 ± 0.07 ^{b} (100)
5 g/ha imazamox	1.50 ± 0.01 ^{b} (84)	0.56 ± 0.01 ^{a} (107)	0.51 ± 0.01 ^{b} (80)	2.70 ±0.05 ^{b} (79)	4.06 ± 0.00 ^{a} (113)
10 g/ha imazamox	1.18 ± 0.07 ^{c} (66)	0.37 ± 0.03 ^{b} (71)	0.51 ± 0.03 ^{b} (80)	3.21 ± 0.15 ^{a} (94)	3.02 ± 0.07 ^{c} (84)
20 g/ha imazamox	1.19 ± 0.08 ^{c} (67)	0.38 ± 0.03 ^{b} (73)	0.52 ± 0.03 ^{b} (81)	3.16 ± 0.06 ^{a} (93)	3.00 ± 0.04 ^{c} (84)

Values followed by different superscript letters (a, b, c) within columns differ significantly at $P<0.05$

The rate of inhibition increases with increasing the herbicide dose from 16% lower Ch *a* content for 5 g/ha imazamox up to 33% for 20 g/ha imazamox.

The gas exchange of maize plants is also suppressed by the herbicide imazamox (Figure 1A). The untreated plants have a net photosynthetic rate of about 11 – 12 µmol CO$_2$/m^2 per sec. The variants received 10 and 20 g/ha of imazamox showed a decrease of 26% and 61% for the treatment in 7-8 leaf stage. The transpiration rate (Figure 1B) is slightly increased by the imazamox treatment with the higher doses of 10 and 20 g/ha.

The maize plants treated with lower (5 g/ha) doses of the herbicide imazamox showed sligth inhibition of growth, where a visible phytotoxicity simptoms such as shlorosis or changes of leaf color were now seen. The plants recieved the higher dose of 10 g/ha showed visible simptoms like chlorosis and more emphasised growth inhibition. These symptoms were overcomed by the plants in different extend and time frame.

The experimental results showed a proportional relationship between increasing the dose of the herbicide imazamox and decreasing the yield of maize plants.

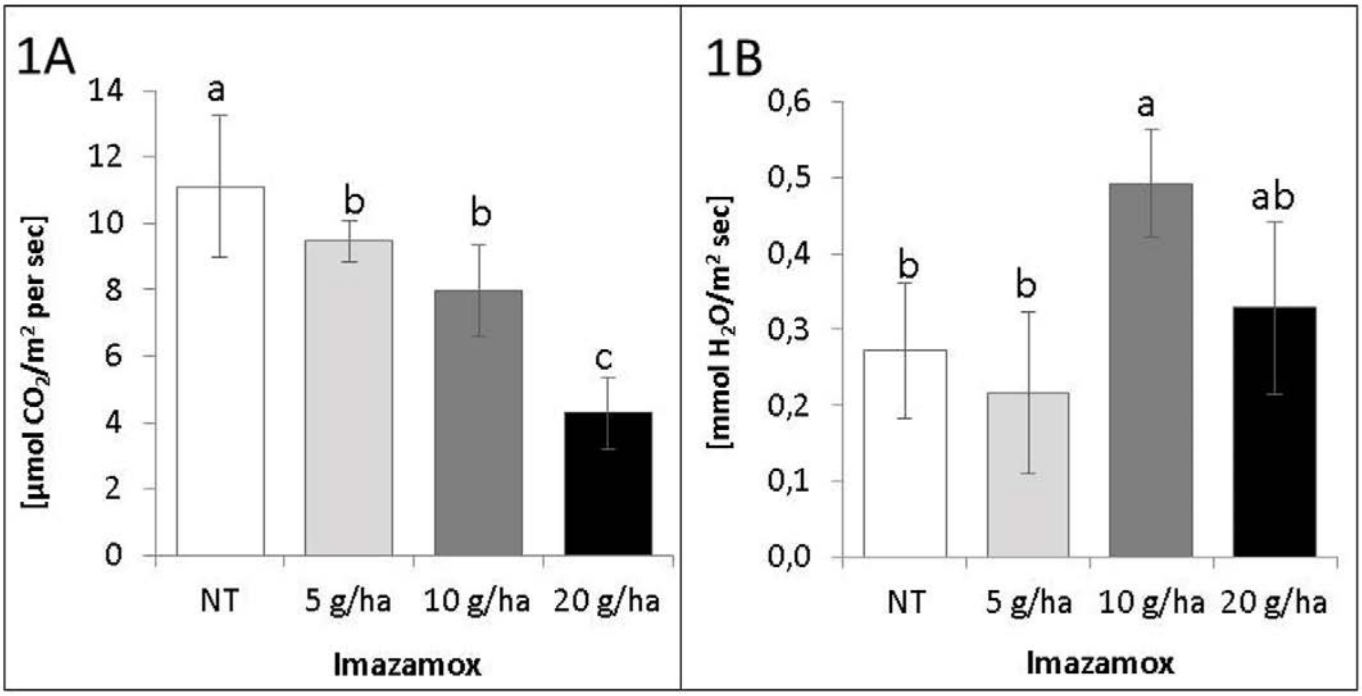

Figure 1. Net photosynthetic rate (1A) and transpiration rate (1B) of maize plants treated with different doses of imazamox. The values represent the mean of five biological replicates, error bars = SD. Different letters (a,b,c) express significant differences ($P<0.05$)

The plants recieved the lower doses recovered faster, with slight productivity decreas, while these treated with the dose of 20 g/ha did not recovered fully and had a significant yield loss. In the variants treated with 10 and 20 g/ha imazamox, the negative effect was expressed in a reduction of the yield (Figure 2) by 15% and 23%, respectively. In the variant treated with 5 g/ha imazamox, no inhibitory effect on yield was observed.

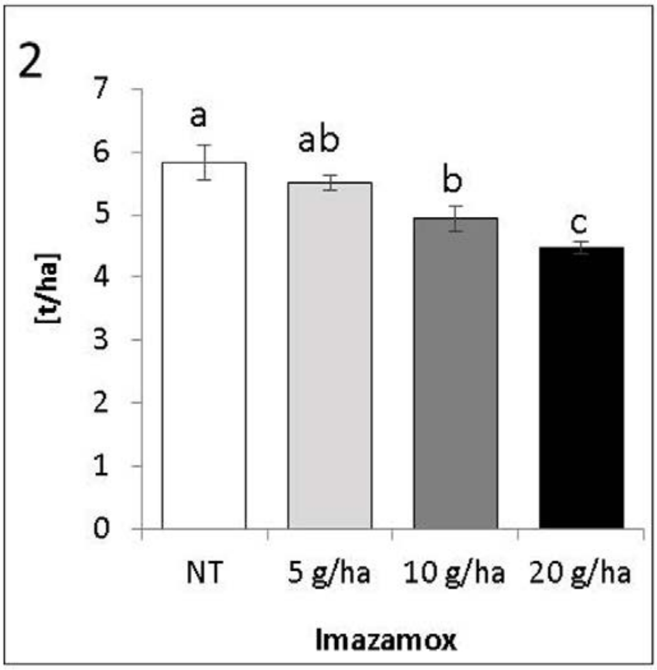

Figure 2. Productivity (t/ha) of maize plants, treated with different doses of imazamox. The values represent the mean of the three replications per treatment, error bars = SD. Different letters (a,b,c) express significant differences (*P*<0.05)

Effects of protein hydrolysates on photosynthetic performance and grain productivity of imazamox-damaged maize plants (experiment 2B)

Based on the results obtained in the range-finding experiment, we selected a recoverable dose, causing moderate phytotoxicity. Therefore we studied the effect of protein hydrolysates with the selected dose of 10 g/ha imazamox. The gas exchange parameters of maize plants (Table 4) also confirm the inhibition caused by a single imazamox treatment with 21% lewer photosynthetic rate. All the variants with the herbicide and PHs showed an ameliorative effect in gas net photosynthesis compared to the single imazamox treatment. The variants treated with imazamox in combinations with various PHs showed significantly improved photosynthetic activity with 6.3%, 8.2% and 15.3% for Naturamin WSP, Terra-sorb and Trainer, respectively. This positive effect caused by the additional biostimulant application was not significant only for the treatment with Naturamin Plus. The transpiration rate of maize plants shows a significant decrease in only for the plants treated with imazamox. In contrast, the untreated control and plants with PHs supply have similar transpiration intensity.

The content of common chlorophylls was determined with the undistructive method by using a portable chlorophyll meter. The results confirm the inhibitory effect of imazamox on maize plants with 19% lower chlorophylls content in imazamox treated variants.

Table 4. Photosynthetic performance of maize plants, treated with imazamox and various protein hydrolysates (A – net photosynthetic rate; E – transpiration rate; Chl. – total chlorophyll content). The values represent the mean of five biological replicates, ± SD. In breckets is presented the percent of the untreated control

Variants	**A** [µmol CO_2/m^2 per sec]	**E** [mmol H_2O/m^2 per sec]	**Chl** [mg/m^2]
Untreated (control)	30.35 ± 0.81 ^{a} (100)	1.25 ± 0.07 ^{a} (100)	363 ± 8 ^{a} (100)
Imazamox (10 g/ha)	23.93 ± 0.42 ^{d} (79)	1.12 ± 0.03 ^{c} (90)	294 ± 11 ^{d} (81)
Imazamox + Naturamin WSP	25.53 ± 1.21 ^{c} (84)	1.24 ± 0.03 ^{ab} (99)	320 ± 5 ^{c} (88)
Imazamox + Naturamin Plus	24.09 ± 0.82 ^{b} (79)	1.2 ± 0.04 ^{b} (96)	345 ± 10 ^{b} (95)
Imazamox + Terra-sorb	26.04 ± 0.41 ^{c} (86)	1.21 ± 0.02 ^{ab} (97)	312 ± 9 ^{c} (86)
Imazamox + Trainer	28.26 ± 1.13 ^{b} (93)	1.23 ± 0.03 ^{ab} (98)	337 ± 9 ^{b} (93)

Values followed by different superscript letters (a, b, c) within columns differ significantly at *P*<0.05

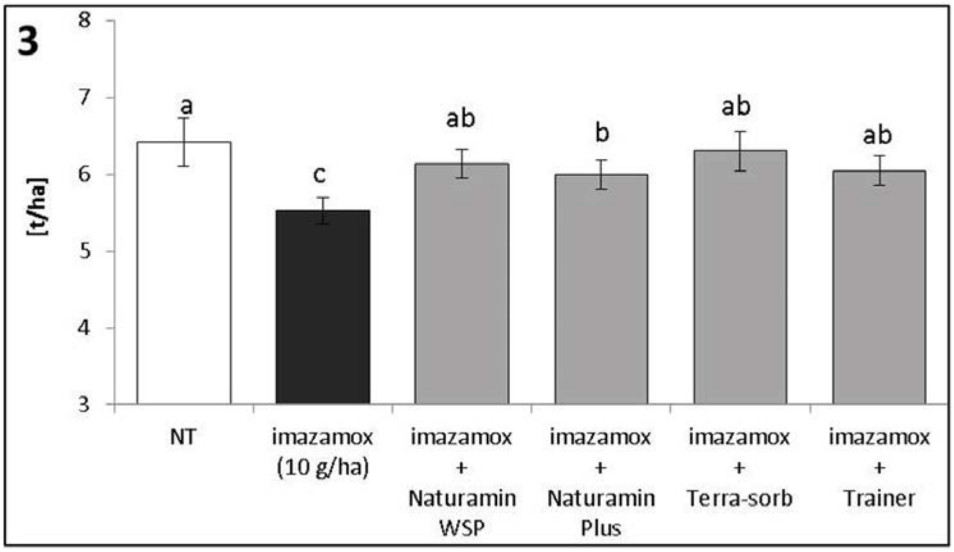

Figure 3. Grain productivity of maize plants, treated with imazamox and various protein hydrolysates. The values represent the mean of the three replications per treatment, error bars = SD. Different letters (a,b,c) express significant differences (*P*<0.05)

The ameliorative effect of PHs is different, varying between 8.3%, 14.8%, 5.1% and 12.6% for variants received application of imazamox and Naturamin WSP, Naturamin Plus, Terra-sorb, Trainer respectively.

The single imazamox treatment significantly reduced the maize productivity (Figure 2) by 16%. The additional application of the PHs had an ameliorative effect on imazamox-damaged maize plants with approximately 10% compared to the imazamox treated plants, where the highest grain productivity is caused by Terra-sorb treatment – 12.4%.

DISCUSSION

According to previous reports, the growth of susceptible plants treated with an imidazolinone herbicide is inhibited within a few hours of application. In comparison, the visual symptoms might be observed within 7 to 14 days as chlorotic meristematic areas and followed by a slow general foliar chlorosis and necrosis (Shaner 1991; Breccia et al. 2011) and reduction in plant fresh, dry weight and leaf area (Bozic et al., 2012). Our results also showed growth arrest of the imazamox treated maize plants, which usually is more pronounced on the plants that received a higher dose of the herbicide. In contrast, doses over 40 g/ha have a lethal effect within a few days after treatment. The chlorosis is directly related to the decreased content of photosynthetic pigments, which could be defined as a typical injury symptom of imidazolinone treatment to susceptible (Hensley et al., 2012; Webster et al., 2016; Garcia-Garijo et la., 2012), but also to resistant crops (Pfenning et al., 2008; Pozniac et al., 2004). In our previous study, we also have found that imazamox may cause changes in photosynthetic pigments profiling even in IMI-R sunflower hybrids (Balabanova et al., 2016; Balabanova et al., 2020).

Similarly, the maize plants treated with different doses of the herbicide imazamox in our experiments also showed symptoms of chlorosis and necrosis mostly at higher doses of treatment, which corresponds with the abovementioned reports (Table 3). Changes in photosynthetic parameters such as chlorophyll fluorescence (Percival and Baker, 1991) and gas exchange (Jimenez et al., 2015; Balabanova and Vassilev, 2015) have been observed in treated plants. A significantly inhibited net photosynthesis of imazamox treated IMI-S maize plants were also found in our study (Figure 1, Table 4), which is in line with other reports concerning the physiological effects of the herbicide. Photosynthesis is not regarded as a primary target of AHAS-inhibiting herbicides, but disorders of the photosynthetic apparatus have been reported many times. This could be the inhibition of the AHAS enzyme activity and subsequent violation of protein turnover. As a result, all the catalytic reactions, including the pigment synthesis and Calvin cycle, are disrupted. The function of proteins taking part in the electron transport chain and transport of photosynthetic products may be impaired.

The injury effect caused by off-target herbicide drift depends on many factors, but mainly on the dose of herbicide received by non-target plants and its sensitivity to the herbicide active ingredients. The imazamox is a selective herbicide, reported as an environmentally friendly compound, but in the case of drift, it has an inhibitory effect on untarget vegetation, including crop species. Deeds et al. (2006) have reported that imazamox applied to wheat at the flowering and jointing growth stages at 33% of the labeled use rate reduced wheat yield by more than 90%. An injury caused by simulated imazamox drift on rice in two phenophases of the plants is reported by Hansley et al. (2012). The author found that necrosis of the flag leaf emerged by 28 days after treatment, resulting in significantly decreased crop yield of 53 and 28% when imazamox was applied to rice at one-tiller and boot, respectively. Webster and co-workers (2016) also report that 66% reduced primary rice yield at the boot timing phase plants received averaged over rate application of imazamox. These data indicate that visual injury to rice is more severe when imazamox is applied during the early vegetative growth stages. Those previous reports also meet the results found in our study, where the herbicide imazamox is causing a significant inhibiting effect on maize grain productivity (Figure 2). This decrease in the productivity of maize plants may be expected since their gas exchange was also decreased and photosynthesis is a process directly related to plant productivity.

Various biostimulants have been available for the last years. A prerequisite for this is that they are defined by a number of improving effects such as enhancement of growth and development by increasing the efficiency of physiological processes within plants and increasing stress tolerance (Nephali et al., 2020). Some studies report on the ameliorative effect of PBs also for herbicide-damaged crops. Soltani and colleagues (2015) report a yield increase of winter wheat and oat after the addition of biostimulants to post-emergence herbicides. Panfili et al. (2019) found that biostimulants containing vitamins, amino acids and proteins reduced the negative effects of metolachlor on maize and increased germination and biomass production. Furthermore, plants treated with the herbicide in combination with Megafol showed lower levels of lipide peroxidation and antioxidant enzymes. In 2018 Andrade and colleagues demonstrated that low doses of glyphosate drift resulted in maize phytointoxication and lower plant height, while hormone-based biostimulant provided increases in stalk diameter, SPAD index and photosynthetic rate. But the report shows that the presence of biostimulant did not provide a significant ameliorative effect on the deleterious effects caused by glyphosate in conventional corn plants. Contrary to the abovementioned reports, Soltani and co-workers (2016) demonstrated that the application of biostimulants containing nutrients and organic acids to the post-emergence herbicides did not affect soybean crop injury and yield.

The protein hydrolysates have been reported to positively impact plants functioning under different abiotic stress conditions, including salinity, heavy metal, thermal, nutrient stress, and water stress (Colla et al., 2017). But the information concerning their impact on herbicide injured crops is far less. An increase in the nitrogen content was found in sunflower plants damaged by imazamox after a protein hydrolysate application (Neshev, 2020). In our previous studies, we have found that some PHs may have an ameliorative effect on imazamox damaged crops such as sunflower, pumpkin, wheat and oilseed rape (Balabanova et al., 2016; Balabanova 2021; Neshev et al., 2020; Neshev et al., 2021a; Neshev et al., 2021b).

The mode of action of the herbicide imazamox is inhibiting the biosynthetic pathway of branched-chain amino acids by blocking the active center acetohydroxyacid synthase (AHAS) (Singh, 1999). We hypothesize that an additional supply of amino acid containing biostimulants, such as PH, could help the imazamox-damaged plants overcome the growth inhibition caused by imazamox. The results confirm the ameliorative effect at about 10% average of the additional treatment with plant biostimulant protein hydrolysate to maize plants damaged by simulated imazamox spray drift on their photosynthetic and transpiration rate, as well as grain productivity. However, the improving effect is not significant for all the evaluated PBs. The products Terra-sorb and Trainer showed a slightly higher ameliorative effect than Naturamin WSP and Naturamin Plus. The ameliorative effect of the PH supply could be due to several reasons, such as the alleviation of protein metabolism by the direct contribution of amino acids into synthesizes of protein molecules and/or the involvement of nutrients in various structures and reactions of a plant cell.

CONCLUSION

The simulated imazamox herbicide drift has a strong inhibiting effect on imizadolinone-susceptible maize plants. This is well illustrated by the growth arrest and disrupted photosynthetic activity of the sprayed plants. The low imazamox dose of 5 g/ha caused a slight injury effect on maize, which plants might overcome. The supply of maize with 10 g/ha imazamox also had an inhibitory but partially recoverable effect. The dose of 20 g/ha had strongly pronounced inhibitory effect on the photosynthesis and growth of maize plant, reduced at the range of 60% and up to 80%, respectively. In comparison, doses over 40 g/ha had a detrimental effect on plant growth and functioning. The additional supply of protein hydrolysates to imazamox damaged maize plants ameliorates plant physiology parameters such as photosynthetic, transpiration rate and content of chlorophylls. As a concequence of the improved physiological state, the grain productivity of imazamox-treated maize plants was also positively influenced up to 14%. Based on our results we may conclude that protein hydrolysate application on maize plants injured by imazamox off-target transfer has improving effect on overall plant performance.

ACKNOWLEDGMENT

This work was funded by Bulgaria National Science Fund (BNSF), in the frame of the project: Agrobiological study on the effect of biostimulants and inorganic crop control products under stress conditions, project number: H16/35.

REFERENCES

Andrade, C. L. L., Carvalho, M. P., Barroso, A. L. L., Rosa, M., Gonçalo, T. P., buchling, C., Rodrigues, R. L. S. (2018) Use of biostimulant in the reversion of injury caused by glyphosate on conventional corn plants. Revista Brasileira de Herbicidas, 17 (4), 1-9.

Balabanova, D. (2021) The ameliorative effect of protein hydrolysate on the imazamox damaged young wheat plants. Agricultural Sciences, 13 (31), 68-76. DOI: https://doi.org/10.22620/agrisci.2021.31.010

Balabanova, D., Vassilev A. (2015) Response of sunflower Clearfield hybrids to both recommendable and higher doses of imazamox herbicide. Agricultural Sciences, 8 (18), 41-46.

Balabanova, D.A., Paunov, M., Goltsev, V., Cuypers, A, Vangronsveld, J., Vassilev, A., (2016) Photosynthetic performance of the imidazolinone resistant sunflower exposed to single and combined treatment by the herbicide imazamox and an amino acid extract. Frontiers in Plant Science, 7. DOI: https://doi.org/10.3389/fpls.2016.01559

Balabanova, D., Remans, T., Cuypers, A., Vangronsveld, J., Vassilev, A. (2020) Imazamox detoxification and recovery of plants after application of imazamox to an imidazolinone resistant sunflower hybrid. Biologia Plantarum, 64, 335-342. DOI: https://doi.org/10.32615/bp.2019.150

Bish, M, Oseland, E, Bradley, K. (2021) Off-target pesticide movement: a review of our current understanding of drift due to inversions and secondary movement. Weed Technology, 35, 345–356. DOI: https://doi.org/10.1017/wet.2020.138

Botta, A. (2013) Enhancing plant tolerance to temperature stress with amino acids: an approach to their mode of action. Acta Horticulturae, 1009, 29–35. DOI: https://doi.org/10.1007/s00299-016-1980-4

Bozic, D., Saric, M., Malidza, G., Ritz, C., Vrbnicanin, S. (2012) Resistance of sunflower hybrids to imazamox and tribenuron-methyl. Crop Protection, 39, 1-10.

Breccia, G., Vega, T., Nestares, G., Mayo, M.L., Zorzoli, R., Picardi, L. (2011) Rapid test for detection of imidazolinone resistance in sunflower (Helianthus annuus L.). Plant Breeding, 130, 109—113. DOI: https://doi.org/10.1111/j.1439-0523.2009.01756.x

Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., Ferrante, A. (2015) Biostimulants and crop responses: a review. Biological Agriculture Horticulture, 31, 1–17. DOI: https://doi.org/10.1080/01448765.2014.964649

Chowdhury, I.F., Doran, G.S., Stodart, B.J., Chen, C., Wu, H. (2020) Trifluralin and atrazine sensitivity to selected cereal and legume crops. Agronomy, 10, 587. DOI: https://doi.org/10.3390/agronomy10040587

Cobb, A. H., Reade J. P. H. (2010) Herbicides and plant physiology (Second edition), West Sussex: Wiley-Blackwell.

Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., Rouphael, Y. (2017) Biostimulant action of protein hydrolysates: unraveling their effects on plant physiology and microbiome. Frontiers in Plant Science, 8. DOI: https://doi.org/10.3389/fpls.2017.02202

de Carvalho, J.P., Nicolai, M., Ferreira, R., Figueira, A.V., Christoffoleti, P.C. (2009) Herbicide selectivity by differential metabolism: Considerations for reducing crop damages. Science Agriculture (Piracicaba, Braz.) 66 (1), 136-142.
DOI: https://doi.org/10.1590/S0103-90162009000100020

Deeds, Z., K. Al-Khatib, D.E. Peterson, Stahlman, P.W. (2006) Wheat response to simulated drift of glyphosate and imazamox applied at two growth stages. Weed Technology, 20, 23-31.

Garcia-Garijo, A., Palma, F., Lluch, C., Tejera, N.A. (2012) Alterations induced by imazamox on acetohydroxyacid synthase activity of common bean (*Phaseolus vulgaris*) depend on leaf position. Pesticide Biochemistry and Physiology, 104, 72–76.

Hensley, J., Webster, E., Blouin, D., Harrell, D., Bond, J. (2012) Impact of drift rates of imazethapyr and low carrier volume on non-clearfield rice. Weed Technology, 26 (2), 236-242.
DOI: https://doi.org/10.1614/WT-D-11-00128.1

Janaki, P., Archana, M., Sathya, Priya, R., Nithya, C., Chinnusamy, C., Prabhakaran, N.K. (2017) Effect of herbicides on potato and their persistence in acid soil under semiarid tropical condition. Advances In Plants And Agriculture Research, 7 (3), 272-277.
DOI: https://doi.org/10.15406/apar.2017.07.00254

Jimenez, F., Fernandez, P., Rojano-Delgado, A.M., Alcantara, R. De Prado R. (2015) Resistance to imazamox in Clearfield soft wheat (*Triticum aestivum* L.). Crop Protection, 78, 15-19.

Jursík, M., Kočárek, M., Kolářová, M., Tichý, L. (2020) Effect of different soil and weather conditions on efficacy, selectivity and dissipation of herbicides in sunflower. Plant, Soil and Environment, 66, 468–476.

Lichtenthaler, H.K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology, 148, 350-382.

Lucini, L., Rouphael, Y., Cardarelli, M., Canguier, R., Kumar, P., Colla, G. (2015) The effect of aplant-derived biostimulanton metabolic profiling and crop performance of lettuce grown under saline conditions. Scienta Horticulturae, 182, 124–133.
DOI: https://doi.org/10.1016/j.scienta.2014.11.022

Nephali, L., Piater, L.A., Dubery, I.A., Petterso, V., Huyser, J., Burgess, K., Tugizimana, F. (2020) Biostimulants for plant growth and mitigation of abiotic stresses: A metabolomics perspective. Metabolites, 10 (12), 505. DOI: https://doi.org/10.3390/metabo10120505

Neshev, N. (2020) Herbicide stress and biostimulant application influences the leaf N, P and K content of sunflower. Scientific Papers. Series A. Agronomy, 63 (2), 172-177.

Neshev, N., Balabanova-Ivanovska, D., Yanev, M., Mitkov, A., Tonev, T. (2020) Effect of biostimulant application in tank mixture with imazamox on common pumpkins (*Cucurbita moschata* Duchesne ex Poir.). Journal of environmental protection and ecology, 21 (5), 1646-1652.

Neshev, N., Balabanova-Ivanovska, D., Yanev, M., Mitkov, A., Tonev T. (2021a) Recovering effect of biostimulant application on pumpkins (*Cucurbita moschata* Duchesne ex Poir.) treated with imazamox. Acta Horticulturae, 1320, 267-274,
DOI: https://doi.org/10.17660/ActaHortic.2021.1320.35

Neshev, N., Balabanova, D., Yanev, M., Mitkov, A., Tonev, T. Vasilev, A. (2021b) Study on biostimulant application at oilseed rape damaged by simulated herbicide drift. Bulgarien Journal of Agricultural Science, 27, 155–160.

Panfili, I., Bartucca, M. L., Marrollo, G., Povero, G., Del Buono, D. (2019) Application of a plant biostimulant to improve maize (*Zea mays*) tolerance to metolachlor. Journal of Agricultural and Food Chemistry, 67 (44), 12164-12171.
DOI: https://doi.org/10.1021/acs.jafc.9b04949

Percival, M.P., Baker N.R. (1991) Herbicides and photosynthesis. In: Baker NR, Percival M.P., eds. Herbicides. Amsterdam: Elsevier, 1–26.

Petrozza, A., Santaniello, A., Summerer, S., Di Tommaso, G., Di Tommaso, D., Paparelli, E. (2014) Physiological responses to Megafol treatments in tomato plants under drought stress: a phenomic and molecular approach. Scienta Horticulturae, 174, 185–192.
DOI: https://doi.org/10.1016/j.scienta.2014.05.023

Pfenning, M., Palfay, G., Guillet, T. (2008) The CLEARFIELD® technology – A new broad-spectrum post-emergence weed control system for European sunflower growers. Journal of Plant Diseases and Protection, 21, 649–653.

Popova, R., Zhalnov, I., Valcheva, E., Zorovski, P., Dimitrova, M. (2012) Estimates of environmental conditions of soils in Plovdiv region in applying the new herbicides for weed control in major field crops. Journal of Central European Agriculture, 12 (3), 595-600.

Pozniak, C.J., Birk, I., O'Donoughue, L.S., Ménard, C., Hucl, P.J., Singh, B. (2004) Physiological and molecular characterization of mutation-derived imidazolinone resistance in spring wheat. Crop Science, 44, 1434-1443.

Sacramento, B.L.D., Azevedo, A.D.D., Alves, A.T., Moura, S.C., Ribas, R.F. (2018) Photosynthetic parameters as physiological indicators of tolerance to cadmium stress in sunflower genotypes. Revista Caatinga, 31, 907–916.
DOI: https://doi.org/10.1590/1983-21252018v31n413rc

Schaafsma, G. (2009) Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition. European Journal of Clinical Nutrition, 63, 1161–1168.
DOI: https://doi.org/10.1038/ejcn.2009.56

Shaner, D.L. (1991) Physiological effects of the imidazolinone herbicides. In: Shaner, D.L., O'Connor S.L., eds. The imidazolinone herbicides. Boca Raton: CRC Press, Inc., 129–137.

Singh, B.K. (1999) Biosynthesis of valine, leucine and isoleucine. In: Singh, B.K., ed. Plant Amino Acids: Biochemistry and Biotechnology. New York: Marcel Dekker, 227–247.

Soltani, N., Shropshire, C. Sikkema, P.H. (2016) Evaluation of biostimulants added to post emergence herbicides in soybean. American Journal of Plant Sciences, 7, 1729-1740.
DOI: https://doi.org/10.4236/ajps.2016.713162

Soltani, N., Shropshire, C., Sikkema, P.H. (2015). Effect of biostimulants added to post-emergence herbicides in corn, oats and winter wheat. Agricultural Sciences, 6, 527–534.
DOI: https://doi.org/10.4236/as.2015.65052

Tomov, T., Rachovski, G., Kostadinova, S., Manolov, I. (2009) Handbook of Agrochemistry. Academic publisher of Agricultural University Plovdiv, 109 pages (in Bulgarian).

Vercampt, H., Vassilev, A., Koleva, L., Horemans, N., Biermans, G., Vangronsveld, J., Cuypers, A. (2016) The functional role of the photosynthetic apparatus in the recovery of *Brassica napus* plants from pre-emergent metazachlor exposure. Journal of Plant Physiology, 196-197, 99-105.

Vieira, B.C.; Luck J.D., Amundsen K.L., Werle R., Gaines T.A., Kruger G.R. (2020) Herbicide drift exposure leads to reduced herbicide sensitivity in *Amaranthus* spp, Scientific Reports, 10, 1-11.

Webster, E., Hensley, J., Blouin, D., Harrell, D., Bond, J. (2016) Rice crop response to simulated drift of imazamox. Weed Technology, 30 (1), 99-105. DOI: https://doi.org/10.1614/WT-D-15-00024.1