

INFLUENCE OF IMAZAMOX ON SOME ANATOMIC INDICES IN THE LEAVES OF SUNFLOWER PLANT (*HELIANTHUS ANNUUS* L.)

*Anastasov H.**

Agricultural University, 12 Mendeleev Str., 4000 Plovdiv, Bulgaria

Received: 29 March 2010 Accepted: 26 May 2010

Summary. The imazamox was applied at a dose of 120ml/da after the process of sunflower seeding. During the vegetation period some visible signs of phytotoxicity in the crop were observed – plant growth inhibition, deformation of leaves and vegetation tip, weak chlorosis, etc. For the purpose of determining the herbicide influence on the sunflower leaf anatomy, several fixed samples from the leaf middle sections were taken as well. The following indices were measured: stomata number (mm²) and stomata size (µm) from the upper and lower epidermis, size of assimilation parenchyma (mesophyll) in leaf. It was established that imazamox caused considerable changes in the sunflower leaf anatomy, which found expression in a reduction of stomata number (mm²) as well as an increase in the thickness of leaf lamina (blade) compared to those in the non-treated control plants.

Key words: imazamox, phytotoxicity, sunflower leaf anatomy, stomata number/mm², mesophyll.

INTRODUCTION

Phytotoxic influence of herbicides is quite various. It is expressed in morphological, anatomical, physiological and biochemical modifications, which occur in sensitive plants and cause deterioration in plants to even lead to death. The biochemical, physiological or anatomical modifications precede the morphological ones. Herbicides reduce the chloroplast content in assimilation parenchyma cells of leaf (Jung et al., 2008), disturb plant biochemical and physiological processes

(Warabi et al., 2001; Ha et al., 2003; Jung et al., 2004; Yang et al., 2006), causing anatomical and morphological modifications (Guh and Kuk, 1997; Kamble, 2007a, b), lead to growth inhibition and death of plants (Martin and Fletcher, 1972; Gorske and Hopen, 1978; Muniyappa et al., 1980; Bakale, 1989; Ferrel et al., 1989; Tripathi et al., 1992; Mukharji, 1994). The objective of the study was to determine imazamox influence on some anatomical indices in the leaves of sunflower plants.

*Corresponding author: hr_anastasov@yahoo.com

MATERIAL AND METHODS

During the period 2006-2007, on the test-field of the Agricultural University, Plovdiv, a field experiment was carried out for the purpose of determining the biological effectiveness and selectivity of some soil herbicides. One of them was the preparation Pulsar 40, with an active substance of 40% imazamox. Imazamox was applied at a dose of 120 ml/da after the process of sunflower seeding. During the vegetation period some visible signs of crop phytotoxicity were observed: plant growth inhibition, deformation of leaves and vegetation tip, weak chlorosis, etc. For the purpose of determining the herbicide influence on sunflower leaf anatomy, several samples from the middle sections of damaged plant leaves as well as from non-treated control plant were taken and fixed in 70% ethanol. A light microscope Amplival was used for the anatomical indices study. The following indices were measured: stomata number (mm^2) and stomata size (μm) from the upper (adaxial) and lower (abaxial) epidermis, as well as the size of assimilation parenchyma (mesophyll) in leaves, upon total zoom of 400x (10x for the ocular and 40x for the lens).

RESULTS AND DISCUSSION

The sunflower leaf (*Helianthus annuus L.*) is dorsiventral. Stomata are located along the both sides of the leaf, which characterize it as amphistomatic. The main epidermal cells are more or less isodiametric with wavy folded and curved anticlinal walls. The stomatal apparatus is of anomocytic (non-row cellular) type, so that the cells around stomata do not differ

from the other basic epidermal cells and the shape of the guard cells is fabaceous. The mesophyll is diverse, represented by palisade (column) and loosely packed (spongy) parenchyma. The palisade parenchyma is one-row and is located directly under the upper epidermis of the leaf. The spongy parenchyma consists of isodiametric scattered parenchyma cells, among which bigger or smaller intracellular spaces are observed. They are often connected to the stomata of the leaf lower epidermis. The influence of imazamox on some anatomical indices in the leaves of sunflower plants is shown in Table 1. The results obtained for the herbicide-treated plants showed a significant increase of the leaf assimilation parenchyma dimensions, which was several times higher than the dimensions reported for the control plants.

The column parenchyma was thicker than the loosely packed parenchyma. The average values obtained for the palisade parenchyma dimensions were $35.83 \mu\text{m}$ for the control plants and $114.6 \mu\text{m}$ for the treated plants, while the spongy parenchyma dimensions were $24.16 \mu\text{m}$ for the non-treated plants and $93.8 \mu\text{m}$ for the treated plants. The significant increase of the assimilation parenchyma dimensions of treated plants was due to the influence of imazamox. Stomata number per mm^2 of the adaxial epidermis was greater than the stomata number of the abaxial epidermis. On the upper epidermis there were (266.6) 368.3 ± 7.12 (441.6) stomata/ mm^2 for control plants and (100) 149.4 ± 5.11 (208.3) stomata/ mm^2 for treated plants, while for lower epidermis stomata number was (258.3) 293.3 ± 4.15 (350) for non-treated plants and (50) 90.27 ± 3.61 (133.3) for treated plants, respectively. The

Table 1. Influence of imazamox on some anatomic indices in the leaves of sunflower plants.

Indexes	Non-treated			Treated		
	(min) $\bar{x} \pm S\bar{x}$ (max)	S [%]	max:min	(min) $\bar{x} \pm S\bar{x}$ (max)	S [%]	max:min
Leaf parenchyma (mesophyll) [μm]						
Palisade parenchyma	(30) 35.83 \pm 0.68 (42.5)	10.41	1.41	(102.5) 114.6 \pm 1.62 (137.5)	7.75	1.34
Spongy parenchyma	(20) 24.16 \pm 0.42 (27.5)	9.54	1.37	(75) 93.8 \pm 1.32 (105)	7.71	1.40
Upper epidermis stomata						
Number/mm ²	(266.6) 368.3 \pm 7.12 (441.6)	10.60	1.65	(100) 149.4 \pm 5.11 (208.3)	18.74	2.08
Length [μm]	(25) 29.75 \pm 0.55 (35)	10.20	1.40	(15) 24.41 \pm 1.07 (40)	24.15	2.66
Width [μm]	(7.5) 20 \pm 0.33 (22.5)	9.30	1.28	(15) 18.33 \pm 0.36 (22.5)	10.94	1.50
Lower epidermis stomata						
Number/mm ²	(258.3) 293.3 \pm 4.15 (350)	7.76	1.35	(50) 90.27 \pm 3.61 (133.3)	21.96	2.70
Length [μm]	(20) 22.7 \pm 0.49 (30)	11.34	1.50	(22.5) 31.5 \pm 0.87 (40)	15.25	1.80
Width [μm]	(15) 17.4 \pm 0.36 (22.5)	11.60	1.50	(10) 24 \pm 0.92 (30)	21.11	3.00

significant reduction of stomata number per area unit in the treated plants led to changes of their dimensions. The length and width (μm) of the adaxial epidermis stomata decreased while the dimensions of abaxial epidermis stomata increased. The changes of stomata dimensions under the influence of imazamox led to atrophy of their guard cells, causing their ineffective functioning. The ineffective functioning of stomata of imazamox-treated leaves along with the significant increase of assimilation parenchyma dimensions and the reduction of stomata number per area unit led to disturbance of the synthetic, transpiration and gas-exchange processes expressed by visible signs of crop phytotoxicity, such as deformation of leaves and vegetation tip, plant growth inhibition, weak chlorosis and subsequent necrotic spots on the leaves of sunflower plants.

CONCLUSIONS

The adverse influence of imazamox on some anatomical indices in the leaves of sunflower plants was expressed by increased dimensions of the assimilation parenchyma in leaves, a reduction of stomata/ mm^2 of leaf upper and lower epidermis and atrophy of stomata guard cells.

REFERENCES

- Bakale VL, 1989. Spray effects of herbicides on *Xanthium strumarium* Linn. The Botanique, 10: 53–65.
- Ferrel MA, TD Whittson, HP Alley, 1989. Control of *Euphorbia esula* with growth regulators herbicide combinations. Weed Technology, 3: 479–484.
- Gorske SF, HJ Hopen, 1978. Effect of two-diphenyl herbicide on *Portulaca oleracea*. Weed Science, 26: 585–588.
- Guh JO, YI Kuk, 1997. Difference in absorption and anatomical responses to protoporphyrinogen oxidase-inhibiting herbicides in wheat and barley. Korean J Crop Sci 42: 68–78.
- Ha SB, SB Lee, DE Lee, OJ Guh, K Back, 2003. Transgenic rice plants expressing *Bacillus* protoporphyrinogen oxidase gene show low herbicide oxyfluorfen resistance. Biol Plant, 47: 277–280.
- Jung HI, YI Kuk, K Back, NR Burgos, 2008. Resistance pattern and antioxidant enzyme profiles of protoporphyrinogen oxidase (PROTOX) inhibitor-resistant transgenic rice. Pesticide Biochem Physiol, 91: 53–65.
- Jung S, Y Lee, K Yang, SB Lee, SM Jang, B Ha, K Back, 2004. Dual targeting of *Myxococcus xanthus* protoporphyrinogen oxidase into chloroplast and mitochondria and high level oxyfluorfen resistance. Plant Cell Environ, 27: 1436–1446.
- Kamble SI, 2007 a. Effect of spray application of oxyfluorfen on anatomical characters of *Hibiscus cannabinus* Linn. Biosci Biotech Res Asia, 4: 671–674.
- Kamble SI, 2007 b. Effect of spray application of 2,4-D on morphological characters of *Hibiscus cannabinus* Linn. Biosci Biotech Res Asia, 4: 705–712.
- Martin JA, JJ Fletcher, 1972. The effect of sublethal doses of various herbicides on lettuce. Weed Res, 12: 268–271.

- Mukharji A, 1994. Effects of certain phenoxy herbicides on mortality, growth and seed output of *Abutilon indicum* (Linn.) S.W. Acta Bot Hung, 38: 335–343.
- Muniyappa TV, TV Ramchandra Prasad, K Krishnamurthy, 1980. Comparative effectiveness of mechanical and chemical method of control of *Parthenium hysterophorus* Linn. Indian J Weed Sci, 12: 137–144.
- Tripathi B, TS Verma, HL Sharma, 1992. Chemical control of *Lantana camera* and its use as organic manure. Indian J Agron Sci, 37: 135–139.
- Warabi E, K Usui, Y Tanaka, H Matsumoto, 2001. Resistance of a soybean cell line to oxyfluorfen by overproduction of mitochondrial protoporphyrinogen oxidase. Pestic Manag Sci, 57: 743–748.
- Yang K, S Jung, Y Lee, K Back, 2006. Modifying *Myxococcus xanthus* proto-porphyrinogen oxidase to plant codon usage and high level of oxyfluorfen resistance in transgenic rice. Pestic Biochem Physiol, 86: 186–194.