Bulg. J. Plant Physiol., 1996, 22(3–4), 66–73

ANTIDOTE EFFECT OF MEIA AGAINST CHLORSULFURON

Georgi Ts. Georgiev *, Ljubomir Iliev, Emanuil Karanov

Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria

Received December 19, 1996

Summary. Greenhouse studies were conducted to evaluate potential herbicide safener for protection of corn from phytotoxic injuries caused by chlorsulfuron [chemical name N^1-(2-chlorosulfonylphenyl)-N^2-(4-methoxy-6-methyl-1,3,5-triazin-5-yl)urea; commersial name – Glean-75, DuPont]. The β-monomethyl ester of itaconic (methylenesuccinic) acid (MEIA) removed considerably the herbicide effect of chlorsulfuron on leaf pigment and protein content, photosynthetic activity and the maize plants height, fresh and dry weight.

Key words: chlorsulfuron, β-monomethyl ester of itaconic acid, herbicide, safener, antidote

Introduction

The wide use of herbicides intended to ensure efficient control of weeds creates significant problems to crop development. The major objective in the science of weed control is to develop selective methods for the control of certain plants (weeds) in mixed populations without injury to the others (crops). One of the ways for solving the problem is the application of selective herbicides. In more cases, this is complicated because some target weeds are botanically related or possess some physiological similarities to the crop being protected. An alternative way to reduce their effect is the treatment of crops with chemically active compounds with antidote effect.

The idea for using herbicidal antidotes belongs to Hoffmann (1962, 1969) who during 60-ies tested and proposed to the practice several preparations. The antidotes are chemical compounds able to alleviate or completely eliminate the phytotoxic ef-

*Corresponding author, FAX 359-(2)-73-99-52, e-mail ALIS@OBZOR.BIO21.ACAD.BG

fects of herbicides on crops without reflecting on their action on weeds. A number of communications are known concerning the antidote effect of different chemicals against thiocarbamate and chloroacetanilide herbicides (Hatzious, 1983, 1984; O'Leary and Prendeville, 1985; Sweetser, 1985; Frear et al., 1987; Gronwald et al, 1987).

In the 80-ies a group of Hungarian scientists gave a proof for antidote activity of a number of dicarboxylic acid derivatives against triazine and chloroacetanilide herbicides (Görog et al., 1982, 1983, 1985). Thus, N-hydroxysuccinimide in the dose of 0.5 kg/ha totally protected sunflower against the phytotoxic effect of N-(3,4)-dichlorophenyl-N′-methyl-N′-methoxyurea. The same compound showed strong antidote effect against the injuries provoked by N-(3-chloro-4-methoxyphenyl)-N,N′-dimethylurea on corn plants. Moreover, endomethylene tetrahydrophtalic acid (2 kg/ha) almost completely suppressed the phytotoxicity of thiocarbamates, ureas, triazines and chloroacetanilides on corn plants.

On the other hand, previous investigations of some authors concerning the regulatory effect of some representatives of dicarboxylic acids, especially β-monomethyl ester of itaconic acid (MEIA), showed that this compound increased leaf pigment content and soluble protein in soybean leaves, enhanced CO_2 fixation and reduced transpiration and stomatal resistance of plants (Velichkov et al., 1989; Georgiev and Karanov, 1990). MEIA also prevented chlorophyll loss in ageing maize leaves, inhibited peroxidase and chlorophyllase activity (Todorov et al., 1992) and increased the efficiency of watering (Todorov and Karanov, 1995). The wide spectrum of MEIA plant growth regulating activity, as well as the results of Hungarian research group (Görog et al., 1982, 1983, 1985) gave us ground to study the possible antidote effect of this compound against herbicide action.

Sulfonylureas are highly active herbicides that possess up to 100 times the activity of many conventional herbicides (Beyer et al., 1988). Because of their high activity and broad spectrum of weed control, sulfonylureas would be a particularly favourable group of herbicides to develop antidotes against. Nevertheless, research on the effect of antidotes on herbicidal activity of sulfonylureas is limited. 1,8-naphtalic anhydride (NA) has good antidote activity against chlorsulfuron [chemical name – N^1-(2-chlorosulfonylphenyl)-N^2-(4-methoxy-6-methyl-1,3,5-triazin-5-yl)urea, commercial name – Glean-75] in corn (Parker et al., 1980; Hatzious, 1984; O'Leary and Prendeville, 1985; Sweetser, 1985; Frear et al., 1987; Devlin and Zbiec, 1990). Hatzious (1984) demonstrated that NA could give good protection and that cyometrinil {(Z)-α-[(cyanomethoxy)imino]benzeneacetonitrile} and {α-[(1,3-dioxolan-2-yl-methoxy)imino]benzeneacetonitrile} could give partial protection to sorghum treated with chlorsulfuron. Sweetser (1985) has shown that ring hydroxylation of chlorsulfuron in corn is enhanced by NA and cyometrinyl.

In the present study, MEIA was tested for its ability to block the phytotoxic activity of chlorsulfuron on corn which is a sensitive crop against this herbicide.

Materials and Methods

The experiments were carried out with maize (*Zea mays* L.) plants, cv. Pioneer grown in greenhouse conditions in pots (20 cm diameter) filled with 10 kg delluvial soil. The plants were regularly irrigated.

The experiments were carried out according the following scheme:

1. Control – seeds were soaked in tap water for 2 h before sowing.
2. Chlorsulfuron (Glean-75, DuPont) – seeds were soaked in 0.1 mM water solution for 2 h before sowing.
3. Chlorsulfuron (seed treatment) + MEIA – spraying of the plants at the 4–5 leaf stage with 7 mM water solution (plus 0.1% Tween-80) till full wetting of the leaves.

The concentration of chlorsulfuron was calculated on base of the dose recommended for practical use (Beyer et al., 1988).

Damages provoked by herbicide treatment was determined visually by using rating scale of 0–3 and leaf necrosis was calculated as a percent (Allison and Watson, 1966).

Leaf pigment content was measured by extraction with 80% acetone, followed by measurement of the optical density at 663, 645 and 460 nm, soluble protein content was determined after the Coomassie Blue dye binding protein method (Bradford, 1976) and photosynthetic activity was determined radiometrically (Yordanov et al., 1969).

All measurements were made at the 8th and 10th leaf stage. Biometrical parameter determinations were made in the 16th leaf stage.

The data presented are averaged from 2 independent experiments, each in 2–4 replications. Results were processed statistically after Fisher.

Results

The first visual symptoms of seed applied chlorsulfuron were retardation of plant growth and leaf necrosis (Fig. 1) The herbicide provoked a strong decrease of leaf pigment content (Fig. 2). Soluble protein content was reduced by 46% in 8th leaf stage and 41% in 10th leaf stage. Intensity of CO_2 fixation was inhibited by 55% (Table 1). Application of MEIA alone did not influence significantly these parameters (data not shown). However, MEIA compensated herbicide phytotoxicity to some extent. Thus, leaf pigment and protein content as well as photosynthetic activity in the treatment with combined application of chlorsulfuron and MEIA showed higher values compared to the treatment with herbicide only. MEIA removed herbicide induced inhibition of protein content with 29% (8 leaf) and 10% (10 leaf). Chlrophyll ($a + b$) con-

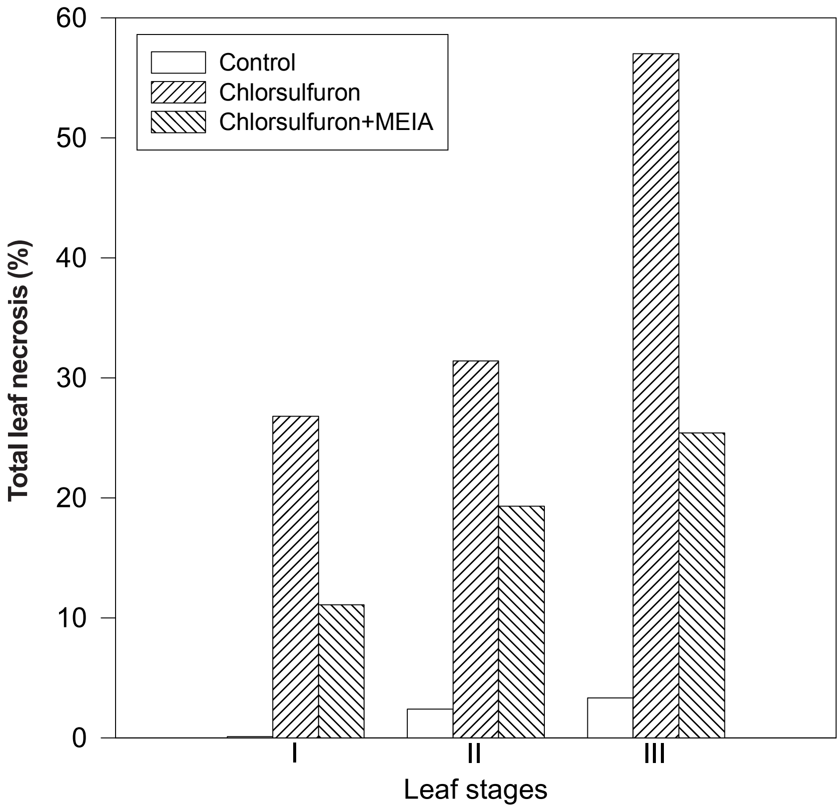

Fig. 1. Percentage of leaf necrosis on maize plants

tent was 17% higher compared to the chlorsulfuron-treated plants. Moreover, MEIA preserved the functional activity of these leaves on a higher level (with 24 and 47%, respectively). However, all values remained significantly lower than the control.

Table 1. Influence of chlorsulfuron and its combinations with MEIA on the protein content and photosynthetic activity of maize leaves. For details of treatments see Materials and Methods

Treatment	Protein content mg/g FW		Photosynthetic activity mg $CO_2/dm^2/h$	
	8 leaf	10 leaf	8 leaf	10 leaf
Control	5.90	2.14	8.01	4.35
Chlorsulfuron	3.19	1.26	3.64	1.96
Chlorsulfuron+MEIA	4.90	1.47	5.51	4.00
LSD 5%	0.76	0.14	1.05	0.97
LSD 1%	1.23	0.37	1.77	1.43

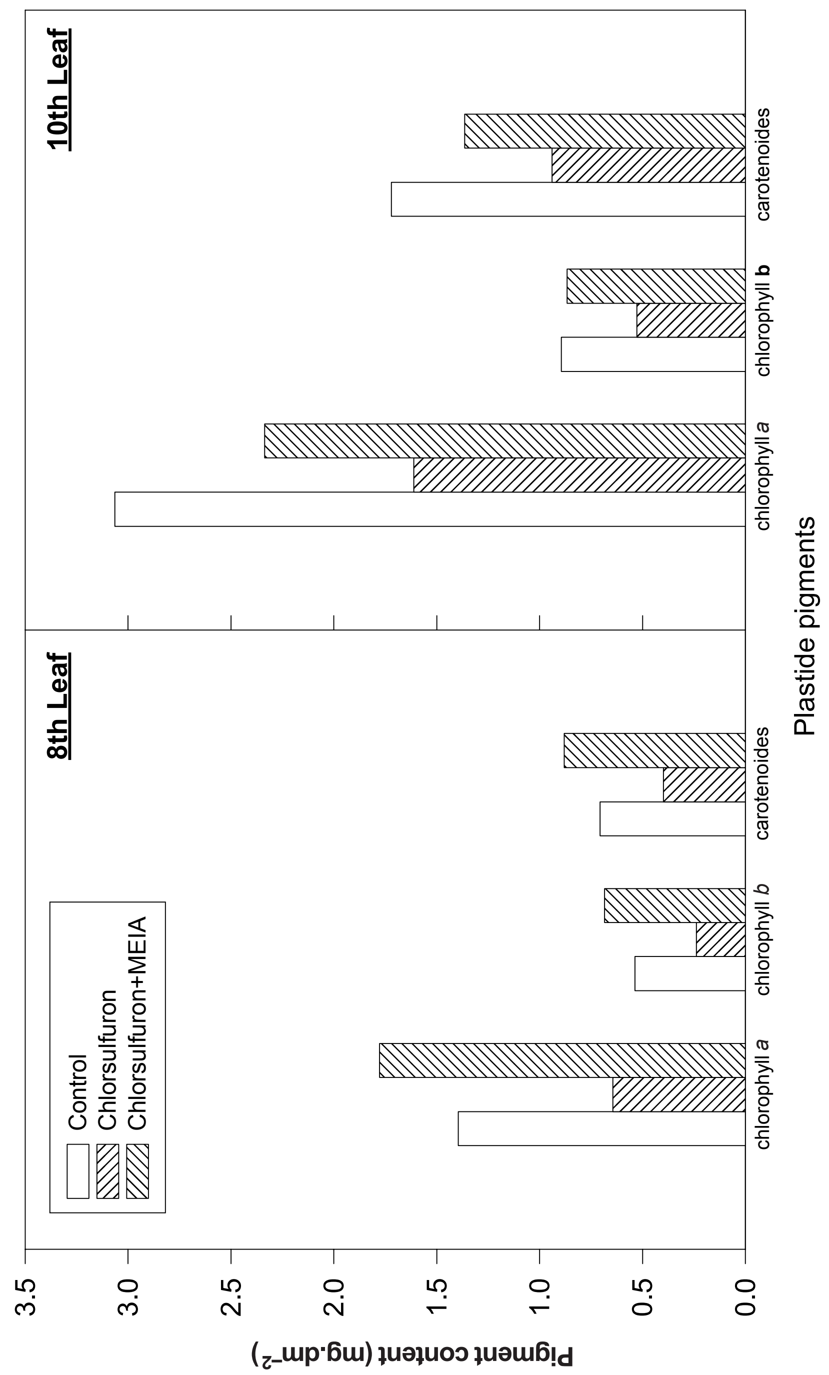

Fig. 2. Effect of chlorsulfuron and its combination with MEIA on leaf pigment content

Table 2. Influence of chlorsulfuron and its combinations with MEIA on the height, fresh and dry weight of maize leaves. For details of treatments see Materials and Methods

Treatment	Plant height cm/plant	Fresh weight g/plant	Dry weight g/plant
Control	191.9	296.4	33.7
Chlorsulfuron	131.4	165.8	19.5
Chlorsulfuron+MEIA	188.9	204.4	28.8
LSD 5%	7.3	14.9	4.4
LSD 1%	11.2	21.5	8.1

At the 16th leaf stage this tendency was preserved (Table 2). Plant height of corn plants treated with 0.1 mM chlorsulfuron were inhibited with 32%. This inhibition was partially abated but not completely overcome by MEIA. As with plant height, decreases in plant fresh and dry weights caused by chlorsulfuron were offset largely by MEIA. Thus, decreases of 44% and 42% in fresh and dry weight provoked by chlorsulfuron were countered in a high degree by MEIA with only an observed 30% loss in fresh weight and a 15% loss in dry weight.

Discussion

Successful use of antidotes to improve crop tolerance to herbicides has been limited mainly to two classes of herbicides – thiocarbamates and chloroacetanilides. How antidotes protect certain grass crops from herbicide injury has not been proven decisively. However, conjugation of glutathione (γ-*L*-glutamyl-*L*-cysteinyl-glycine) with the sulfoxides of thiocarbamates and with chloroacetanilide herbicides, rendering these herbicides inactive, has been demonstrated (Lay and Casida, 1976; Leavitt and Penner, 1979; Lamoureux and Rusness, 1983; Fuerst and Gronwald, 1986; Cobb, 1992). Metabolism of these herbicides in this manner is enhanced by herbicide antidotes such as flurazole [phenylmethyl 2-chloro-4-(trifluoromethyl-5-thiazolecarboxylate] and dichlormid (2,2-dichloro-N,N-di-2-propenylacetamide) which raise the level of glutathione in the plants (Mozer et al., 1983; Gronwald et al., 1987).

Whether MEIA, a possible herbicide antidote, raises the level of glutathione or glutathione-S-transferase in the plants is unknown. However, Görog et al. (1982, 1983, 1985) have shown that other derivatives of dicarboxylic acids increase corn shoot and root tolerance to both thiocarbamate and chloroacetanilide herbicides. It seems probable that the antidote activity of MEIA is similar to that of other antidotes such as flurazole and dichlormid. However, MEIA could also act on the other endogenous plant defence systems – ascorbate/dehydroascorbate ratio, polyamine content, activity of catalase, peroxidase, superoxide dismutase etc. How this compound realises its protective action against chlorsulfuron in maize plants remains to be elucidated.

Acknowledgements: This study was supported by Grants K-424/94 and B-413/94 from the National Science Fund, Bulgaria.

References

Allison, C., D. I. Watson, 1966. The production and distribution of dry matter in maize after flowering. Ann. Rev. Bot., 30, 361–365.

Bradford, M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248–254.

Beyer, E., M. Duffy, J. Hay, D. Schueter, 1988. Sulfonylureas. In: Herbicides. Chemistry, Degradation and Mode of Action. Eds. P. Kearney and D. Kaufman, Vol. 3. Marcel Dekker Inc., NY, pp. 117–189.

Cobb, A., 1992. Herbicides and Plant Physiology. Chapman & Hall, London-NY-Tokyo-Melbourne-Madras.

Devlin, R., I. Zbiec, 1990. Effect of BAS-145-138 as an antidote for sulfonylurea herbicides. Weed Technol., 4, 337–340.

Frear, D., H. Swanson, D. Mansager, 1987. 1,8-Naphtalic anhydride/auxin protection against chlorsulfuron inhibition of corn seedlings growth. In: Pesticide Science and Biotechnology, Eds. R. Greenhalgh and T. Roberts, Blackwell Scientific Publ., Oxford, 161–168.

Hatzious, K., 1983. Herbicide antidotes: development, chemistry, and mode of action. Adv. Agron., 36, 265–315.

Hatzious, K., 1984. Potential safeners for protecting sorghum (*Sorghum bicolor* L. Moench) against chlorsulfuron, fluazifop–butyl and sethoxydim. Weed Res., 24, 249–254.

Hoffman, O. L., 1962. Chemical seed treatments as herbicide antidotes. Weed. Sci., 10, 322–328.

Hoffman, O., 1969. Chemical antidotes for EPTC on corn. Weed Sci. Soc. Am. Abstr., 12–125.

Georgiev, G. Ts., E. Karanov, 1989. IFR-13 – a biologically active preparation for increasing the quality and productivity of soybean (*Gl. max* M.) plants. FECS Intern. Conf. on Chem. and Biotechnol. of Biol. Active Natural Products, Vol. 4, 356–360.

Görog, K., D. Erzsebet, G. Ivan, T. Marta, 1982. Herbicide antidote for plants. Pat. Austria No368677, CA 1983, v. 98, 48678p.

Görog, K., S. Goal, D. Erzsebet, K. Jeno, G. Ivan, T. Marta, 1983. Dicarboxylic acid antidotes for triazine herbicides. Pat. Austria No532511, CA 1984, v. 100, 134305.

Görog, K., E. Sandor, D. Erzsebet, K. Jeno, G. Ivan, T. Marta, 1985. Dicarboxylic acid antidotes for chloroacetoanilide herbicides. Pat. Hungary No178891, Ref. J. Chemie 1985, 70473 P.

Gronwald, J., E. Fuerst, C. Eberlein, M. Egli, 1987. Effect of herbicide antidotes on glutathione content and glutathione-S-transferase activity of sorghum shoots. Pestic. Biochem. Physiol., 29, 66–76.

Lamoureux, G., D. Rusness, 1983. Malonylcysteine conjugates as end products of glutathione conjugate metabolism in plants. In: IUPAC Pesticide Chemistry: Human Welfare and the Environment, Eds. J. Miamoto and P. Kearney, Pergamon Press, NY, pp 295–300.

Lay, M., E. Casida, 1976. Dichloroacetoamide antidotes enhance thiocarbamate sulfoxide detoxification by elevating corn root glutathione content and glutathione-S-transferase activity. Pestic. Biochem. Physiol., 6, 442–456.

Leavitt, J., D. Penner, 1979. In vitro conjugation of glutathine and other thiols with acetanilide herbicides and EPTC sulfoxide and the action of the herbicide antidote R-25788. J. Agric. Food. Chem., 27, 533–536.

Mozer, T., D. Tiemeir, E. Jaworski, 1983. Purification and characterization of corn glutathione-S-transferase. Biochem., 22, 1068–1072.

O'Leary, N., V. Prendeville, 1985. Uptake and phytotoxicity of chlorsulfuron in *Zea mays* L. in the presence of 1,8-naphtalic anhydride. Weed Res., 25, 331–339.

Parker, C., W. Richardson, T. West, 1980. Potential for expending the selectivity of DPH-4189 by use of herbicide safeners. Proc. Br. Crop Prot. Conf. Weeds, 1, 15–21.

Sweetser, P., 1985. Safening of sulfonylurea herbicides to cereal crops: Mode of herbicide antidote action. Proc. Br. Crop Prot. Congr. Weeds, 3, 1147–1154.

Todorov, D., V. Alexieva, E. Karanov, D. Velichkov, V. Velikova, 1992. Effect of certain dicarboxylic acid monoesters on the growth, chlorophyll content, chlorophyllase and peroxidase activities and gas-exchange of young maize plants. J. Plant Growth Regulation, 11, 233–238.

Todorov, D., E. Karanov, 1995. Changes in mineral content of young maize plants under the influence of some dicarboxylic acid monoesters. J. Plant Nutrition, 18(1), 25–34.

Velichkov, D., G. Ts. Georgiev, Ts. Tsonev, E. Karanov, 1989. Influence of some esters of dicarboxylic acids on photosynthesis and transpiration of soybean plants. Bulg. J. Plant Physiol.(Sofia), 15(4), 21–26 (In Bulg.).

Yordanov, I., K. Popov, P. Chichev, 1969. Radiometrical determination of intensity of photosynthesis by ^{14}C. Bulgarian National Isotope Conference, 1969.