SHORT COMMUNICATION

Interaction between Chlorsulfuron and Phenylurea Cytokinin 4-PU-30 on Resistant and Susceptible Plants*

GEORGI GEORGIEV and LUBOMIR ILIEV

M. Popov Institute of Plant Physiology, Bulgarian Academy of Science, Sofia, Bulgaria

Abstract

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Laboratory studies determined the effectiveness of the phenylurea cytokinin 4-PU-30 as protectant of corn (*Zea mays* L.) against postemergence chlorsulfuron injury. The effects of herbicide and cytokinin were also studied on wheat (*Triticum aestivum* L.), a species that is resistant to chlorsulfuron. 4-PU-30 and chlorsulfuron had opposite effects on the growth of corn and wheat plants: 4-PU-30 stimulated the growth of corn and suppressed that of wheat, whereas chlorsulfuron stimulated wheat growth and inhibited that of corn. Chlorsulfuron overcame the inhibition of wheat seedlings caused by 4-PU-30, and 4-PU-30 protected corn from postemergence applied sulfonylurea herbicide chlorsulfuron; moreover in some cases a stimulated growth of the seedlings was registered. The results indicated that 4-PU-30 acted as an antidote against chlorsulfuron on corn.

Key words: corn; wheat; herbicide antidote, sulfonylurea, phenylurea cytokinin

Sulfonylureas are highly active herbicides that possess up to 100 times the activity of many conventional herbicides. Because of their high activity and broad spectrum of weed control, the sulfonylureas would be a particularly suitable group of herbicides to develop antidotes against. However, research on this subject is limited.

The first commercialized chlorsulfuron is effective on a broad spectrum of weeds, and is safe to use on such small grains cereals such as wheat, barley, oats and rye. Use rates as low as 4–5 g a.i./ha control important broadleaf weeds and several grasses in these crops. Corn (*Zea mays* L.), one of the most important crops in plant production, is sensitive to chlorsulfuron. PARKER *et al.* (1980) were the first to observe a protecting effect of naphthalene-1,8-dicarboxilic acid anhydride on corn. Other data indicated that the herbicide antidote BAS-145-138/1 – dichloro-acetylhexahydro-3,3,8-α-trimethyl-pyrrolo-(1,2-α)-pyrimidin-6-(2H)-one blocks almost completely the phytotoxic activity of chlorsulfuron on corn (DEVLIN & ZBIEK 1991). Cytokinins of the purine and phenylurea type regulate in a similar way the growth and physiological processes (MOK 1994). However, phenylurea derivatives have a higher activity at relatively low concentrations and are an excellent basis for application in plant production. Protection against chilling or freezing after treatment with kinetin and 4-PU-30 (N-(2-chloro-4-pyridyl)-N´-phenylurea) on different cultivated plants such as marrows, tomatoes, cucumber, wheat and tobacco tissue cultures, has been demonstrated in growth chamber and greenhouse experiments (ILIEV *et al.* 1994). We have shown recently (GEORGIEV & ILIEV 1996) that the seed treatment of corn with 4-PU-30 stimulated the early growth of seedlings in light.

The combined application of certain herbicides with plant growth regulators could bring about synergistic effects useful for weed control or plant growth regulation.

The above mentioned growth-regulating and protecting properties of 4-PU-30 on different plant species, including wheat and corn (resistant and sensitive to chlorsulfuron), were the reasons to initiate this study.

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MATERIAL AND METHODS

Wheat cultivar Sadovo 1 and corn cultivar Kneza 650 were used for the experiments. The seeds were soaked for 5 h in different concentrations of 4-PU-30; control seeds were soaked in tap water. The treated seeds were put in Petri dishes on two layers of filter paper and placed in the dark for 48 h at 22 ± 1°C. After germination the seedlings were arranged at the holes in covers of pots (15 seedlings per pot) containing water solutions of chlorsulfuron, and exposed for 14 d at 20 ± 1°C under constant illumination (160 mmol/m^2/s, 16 h light and 8 h dark). The control seedlings were grown on tap water. After 14 day the seedlings were removed from the pots and the roots thoroughly washed and dried. The 10 largest seedlings (determined by shoot length) were selected for measurement. Their shoot and root lengths, fresh and dry weights were determined. The data presented are the means of two experiments with six replications in each. The data are expressed as the average length, shoot fresh and dry weight per plant. The experimental values were analysed statistically. The least significant deviation (LSD) was used to evaluate differences between the variants according STEEL and TORRIE (1960).

RESULTS

Influence of 4-PU-30

Wheat: Weak inhibition of stem growth was observed under the influence of the highest concentration of 4-PU-30 at 1.2.10^{-4} M. Growth of the root system was suppressed by all treatments, and most by the highest concentration of 4-PU-30 (Table 1). Shoot fresh weight was not considerably affected by the treatment, whereas dry weight was reduced about 20% by the concentrations used. The fresh and dry weight of roots was strongly reduced in seedlings developed from seeds treated with 4-PU-30 causing decreased root length. Lower fresh and dry weight of shoots was mostly caused by the highest concentration, at 1.2.10^{-4} M.

Corn: 4-PU-30 stimulated the length of the shoots by 12% when treated with 8.10^{-5} M, and 8% when seeds were soaked in 1.2.10^{-4} M (Table 2). All treatments increased the fresh weight, but at the same time dry weight was reduced. Root length was not influenced considerably, but a slight increase of growth, fresh and dry weight was registered when treated with 8.10^{-5} M.

2. Influence of chlorsulfuron

Wheat: Chlorsulfuron increased shoot length by 23% when treated with 10^{-6} M (Table 1). Fresh and dry weights exceeded those of the untreated control. The high herbicide concentration of 10^{-3} M had no influence on seedlings growth, fresh and dry weight. Root length was increased (24 %) when treated with 10^{-6} M, and dry weight reduced at 10^{-3} M.

Corn: Chlorsulfuron reduced the length of shoots and roots of corn seedlings, and this reduction increased with the concentration (Table 2). The same trend was evident in dry weight of shoots and fresh weight of roots.

Table 1. Interactions between chlorsulfuron and 4-PU-30 on wheat

Variants	Shoots						Roots					
	length (mm)	%	fresh weight (mg/plant)	%	dry weight (mg/plant)	%	length (mm)	%	fresh weight (mg/plant)	%	dry weight (mg/plant)	%
Control (H_2O)	204	100	193	100	21.8	100	229	100	165	100	15.6	100
1.2.10^{-4} M 4-PU-30	185	91	195	101	17.5	80	135	59	93	56	6.5	42
8.10^{-5} M 4-PU-30	211	103	238	123	19.3	86	170	74	118	72	9.7	62
4.10^{-5} M 4-PU-30	202	99	198	102	17.4	80	162	71	118	72	9.4	60
4.10^{-3} M Chlorsulfuron	216	106	195	101	20.4	93	225	98	104	93	11.2	72
4.10^{-6} M Chlorsulfuron	252	123	214	111	23.9	109	285	124	161	98	12.1	78
1.2.10^{-4} M 4-PU-30 + 10^{-3} M Chlorsulfuron	175	86	152	79	18.5	85	137	60	95	58	8.2	53
1.2.10^{-4} M 4-PU-30 + 10^{-6} M Chlorsulfuron	183	90	175	91	20.3	93	155	68	113	68	8.5	54
8.10^{-5} M 4-PU-30 + 10^{-3} M Chlorsulfuron	179	88	176	91	18.5	85	198	86	155	94	11.3	72
8.10^{-5} M 4-PU-30 + 10^{-6} M Chlorsulfuron	190	93	184	95	20.9	96	207	90	160	97	11.3	72
4.10^{-5} M 4-PU-30 + 10^{-3} M Chlorsulfuron	192	94	174	90	18.8	86	199	87	153	93	11.7	75
4.10^{-5} M 4-PU-30 + 10^{-6} M Chlorsulfuron	195	96	189	98	20.5	94	206	90	159	96	12.4	79
LSD 5%	8.1		12.7		3.4		11.8		10.3		3.1	
1%	13.4		17.3		4.6		16.2		13.9		4.4	

Table 2. Protective influence of 4-PU-30 on the growth of corn against the effect of the herbicide chlorsulfuron

Variants	Shoots length (mm)	%	Shoots fresh weight (mg/plant)	%	Shoots dry weight (mg/plant)	%	Roots length (mm)	%	Roots fresh weight (mg/plant)	%	Roots dry weight (mg/plant)	%
Control (H_2O)	265	100	381	100	26.0	100	171	100	238	100	21.2	100
$1.2.10^{-4}$ M 4-PU-30	286	108	449	118	17.9	69	149	87	221	93	18.9	89
8.10^{-5} M 4-PU-30	296	112	468	123	22.9	88	185	108	269	113	22.0	104
4.10^{-5} M 4-PU-30	273	103	419	110	18.5	71	171	100	243	102	21.6	102
4.10^{-3} M Chlorsulfuron	172	65	331	87	18.7	72	126	73	133	56	18.5	87
4.10^{-6} M Chlorsulfuron	195	73	355	88	22.3	86	138	81	166	70	19.2	90
$1.2.10^{-4}$ M 4-PU-30 + 10^{-3} M Chlorsulfuron	243	92	422	111	25.0	96	112	65	207	87	21.8	103
$1.2.10^{-4}$ M 4-PU-30 + 10^{-6} M Chlorsulfuron	223	84	354	93	26.7	103	148	86	172	72	21.5	101
8.10^{-5} M 4-PU-30 + 10^{-3} M Chlorsulfuron	274	103	565	148	28.0	108	178	104	234	98	19.7	93
8.10^{-5} M 4-PU-30 + 10^{-6} M Chlorsulfuron	286	108	613	160	28.3	109	168	98	206	87	19.0	90
4.10^{-5} M 4-PU-30 + 10^{-3} M Chlorsulfuron	235	89	404	106	27.4	105	165	96	226	95	17.5	83
4.10^{-5} M 4-PU-30 + 10^{-6} M Chlorsulfuron	278	105	509	133	27.9	107	169	99	245	103	18.4	87
LSD 5%	18.5		37.4		2.9		16.2		20.8		3.8	
1%	25.5		50.2		4.1		22.2		27.6		5.2	

3. Combined treatment

Wheat: The combined treatment of 4-PU-30 with chlorsulfuron reduced shoot growth about 10%, which could be considered to offset the stimulating action of chlorsulfuron (Table 1). A similar trend was observed in shoot fresh weight. Dry weight was reduced 20% by the phenylurea cytokinin; this reduction was partially offset by the combined treatment. The inhibition of root growth by 4-PU-30 at 4.10^{-5} and 8.10^{-5} M was to a certain extent compensated by chlorsulfuron. The reduction of shoot length and dry weight due to the cytokinin were also compensated to a certain extent by the herbicide.

Corn: Corn plants treated with 10^{-3} M and 10^{-6} M of chlorsulfuron showed a reduction of shoot length of about 12–13%. This reduction was completely compensated by all rates of 4-PU-30. Moreover, a trend for growth stimulation by the lower concentrations of 4-PU-30 was observed. Fresh weight recovery was more pronounced, especially at 8.10^{-5} M of 4-PU-30. Reduced root growth caused by chlorsulfuron was to a certain extent negated by 4.10^{-5} M and 8.10^{-5} M of 4-PU-30. The reduction of root fresh weight reduction by the herbicide was similarly negated by the same rates of the phenylurea cytokinin. A slight increase in root dry weight was observed in seedlings developed from seeds treated with $1.2.10^{-4}$ M of 4-PU-30 and additionally grown on herbicide solution.

All values of shoot and root length, fresh and dry weight of corn seedlings treated with chlorsulfuron indicated the protective effect of the phenylurea cytokinin 4-PU-30.

DISCUSSION

Chlorsulfuron and 4-PU-30 had reverse effects on the growth of wheat and corn plants. Chlorsulfuron stimulated growth of the species resistant to it (wheat) and inhibited that of the sensitive one (corn), whereas 4-PU-30 enhanced growth of corn that is sensitive to the herbicide and inhibited growth in the resistant wheat (Tables 1 and 2). We can assume that the inhibitory action of 4-PU-30 on wheat seedlings in light involves a blockage of electron transport between Q (primary electron acceptor for PSII) and PQ (plastoquinone) or acts as a herbicide of diurone type. The similar structure of 4-PU-30 and urea herbicides (urea bridge as a common side) motivated this assumption. We have shown recently increased dry weight, storage proteins, chlorophyll content and CO_2-fixation after leaf treatment of corn seedlings (STEFANOV *et al.* 1994). Other results indicated stimulated growth of corn seedlings after seed soaking in 10–30 mg/l solution of 4-PU-30 (GEORGIEV & ILIEV 1996). The data presented in Table 2 support these results: after treatment of corn seeds with 8.10^{-5} M and $1.2.10^{-4}$ M 4-PU-30 the shoot length and fresh weight exceeded considerably those of the control plants, respectively 12–23% and 8–18%. The stimulating effect of 4-PU-30 on the growth of corn seedlings is probably due to its property to enhance some enzyme activities, and protein and chlorophyll synthesis (STEFANOV *et al.* 1994). On the other hand, some of the main cytokinin properties are well known: stimulation of seed germination and early seedlings growth, nucleic acids and protein syntheses, chlorophyll accumulation etc. A concentration of 10^{-6} M of chlorsulfuron enhanced the growth of wheat seedlings (Table 1). This stimulation could be explained with its metabolism in the resistant plant which occurs via hydroxylation on the phenyl ring, followed by conjugation with glucose, and also with its cytokinin properties when used in low concentrations. The exact mechanism of action of sulfonylurea herbicides are not complectely understood. It is well known that sulfonylureas block the activity of acetolactate synthase (ALS), an enzyme which is necessary for synthesis of the branched essential amino acids valine, leucine and isoleucine. Thus, depletion of the amino acid pool results in a decrease in cell cycle-specific proteins or nucleic acids. It has been also proposed that accumulation of the intermediate 2-ketobutyrate rather than depletion of branched amino acids inhibits growth events (LA ROSSA *et al.* 1987). Unfortunately, there are no data in the literature concerning the mode of action of 4-PU-30 as an inhibitor of plant growth.

The combination of 4-PU-30 and chlorsulfuron in wheat (a plant resistant to chlorsulfuron but susceptible to 4-PU-30) caused inhibition of seedlings growth and dry weight accumulation of about 10%. These results indicate that the interactive effects between 4-PU-30 and chlorsulfuron were antagonistic. At the same time, chlorsulfuron protected wheat roots against 4-PU-30. This means that the herbicide acts as a safener against 4-PU-30 in wheat. The strong reduction by corn (a plant sensitive to the herbicide) was counteracted by the phenylurea cytokinin at all application rates, moreover, a trend to enhanced growth was observed. Obviously the phenylurea cytokinin 4-PU-30 protects corn against the phytotoxic effects of chlorsulfuron. Or the seed treatment with cytokinin increased to a great extent the tolerance of corn seedlings against the phytotoxicity caused by the postemergence herbicide application. As mentioned above, the antidote activity on corn plants against chlorsulfuron is mainly observed in substances which are not plant growth regulators (PARKER *et al.* 1980). Good results were also obtained by using the safener BAS-145-138, which has a structure similar to those of some pyrolo-pyrimidines known as cytokinin antagonists (IWAMURA 1994). The exact mechanisms through which the currently available herbicide safeners protect grass crops against sulfurylureas including chlorsulfuron are not fully understood. R-25788 is supposed to act as a safener of thiocarbamate herbicides on corn either by counteracting substance phytotoxicity through a competitive inhibition at some common site within the protected plant (EZRA *et al.* 1982) or by stimulating the detoxication of these herbicides in the protected plant tissues (KOMIVES & DUTKA 1980). It is hardly probable to suppose a similar mode of action of the above mentioned antidote against thiocarbamates on corn and the protecting action of 4-PU-30 on corn against chlorsulfuron. However, the similar structure of chlorsulfuron and 4-PU-30 (occurrence of the aryl group, urea bridge and a nitrogen-containing heterocycle in both substances) allows to suppose a common site - a probable replacement of the herbicide molecule by the cytokinin moiety from the receptor site. STEFANOV *et al.* (1994) demonstrated higher protein and chlorophyll content, increased dry weight and CO_2-fixation in leaves of corn seedlings treated with 4-PU-30. Stimulated growth after seed treatment with the same substance has been also observed (GEORGIEV & ILIEV 1996). It may be assumed that the disturbed protein metabolism and inhibited growth of corn seedlings after chlorsulfuron treatment were countered to a certain extent by the stimulating action of 4-PU-30 on these processes. An enhancement of ALS activity which intensifies the herbicide degradation in plant tissue could not be excluded.

The results of this study suggest that phenylurea cytokinin 4-PU-30 could serve to protect corn from the phytotoxic effects of residual amounts of sulfonylurea herbicides in fields where a wheat/corn rotation takes place. Moreover, dressing of the corn seeds with 4-PU-30 is an easy approach and shows good results (GEORGIEV & ILIEV 1996).

However, further research is needed to elucidate the postulates mentioned above.

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Souhrn

GEORGIEV G., ILIEV L. (2000): **Interakce chlorsulfuronu a fenylurea cytokininu 4-PU-30 na rezistentních a citlivých rostlinách**. Plant Protec. Sci., **36**: 107–111.

Laboratorní studie prokázaly účinnost fenylurea cytokininu 4-PU-30 při ochraně kukuřice (*Zea mays* L.) proti postemergentnímu poškození chlorsulfuronem. Účinky herbicidu a cytokininu byly také studovány na pšenici (*Triticum aestivum* L.), druhu, který je rezistentní vůči chlorsulforonu. 4-PU-30 a chlorsulforon měly opačné účinky na růst kukuřice a pšenice: 4-PU-30 stimuloval růst kukuřice a potlačil růst pšenice, zatímco chlorsulforon stimuloval růst pšenice a potlačil růst kukuřice. Chlorsulforon zabránil poškození klíčních rostlin pšenice způsobenému 4-PU-30 a ochraňoval kukuřici po postemergentní aplikaci chlorsulfuronu ze skupiny sulfonylmočovin; v některých případech však byla zjištěna stimulace růstu klíčních rostlin. Z výsledků vyplynulo, že 4-PU-30 působí jako antidot chlorsulfuronu u kukuřice.

Klíčová slova: kukuřice; pšenice; antidot herbicidu; sulfonylmočovina; fenylurea cytokinin

Corresponding author:

Dr. GEORGI GEORGIEV, Bulgarien Academy of Sciences, Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bt. 21, 1113 Bulgaria, tel.: + 359 2 97 93 675, fax: + 359 2 73 99 52, e-mail: iliev@obzor.bio21.bas.bg