RESPONSE OF MAIZE (*ZEA MAYS* L.) TO RIMSULFURON UNDER SALT CONDITIONS

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ABSTRACT

Investigations were carried out to determine the possible interaction between salinity (60 mmol·dm^{-3} NaCl) and phytotoxicity of herbicide Titus 25 DF (sulfonylurea herbicide containing active ingredient rimsulfuron). The herbicide was added to the nutrient solution at two concentrations – 1 or 100 nmol·dm^{-3}. After 7 days of cultivation in the nutrient solution determined were the growth parameters and some biochemical compounds (photosynthetic pigments, total protein and free amino compounds content). The combined action of 1 nmol·dm^{-3} rimsulfuron and salt caused a reduction in plant biomass accumulation, but this reduction was due to the salt itself. In the presence of 100 nmol·dm^{-3} rimsulfuron, growth inhibition of maize roots was very high and salinity did not modify herbicide toxicity. However, reduction in maize shoots growth equalled the sum of the reductions caused by each particular factor applied separately (additive effect). The reduction in shoot fresh weight reached 64%.

The investigated stress factors induced a significant increase in amino compounds, with the exception of maize roots grown under NaCl plus 100 nmol·dm^{-3} rimsulfuron. The concentration of total protein in maize roots diminished under NaCl and 100 nmol·dm^{-3} rimsulfuron, although in the other cases, it did not change distinctly in comparison to the control. All factors, with the exception of NaCl, induced a slight increase in protein contents in maize leaves. Stress factors did not change significantly the total chlorophyll concentration, however, carotenoid content was markedly reduced. Nevertheless, the combined action of 100 nmol·dm^{-3} rimsulfuron and NaCl caused a 10% increase in carotenoid content as compared to the control plants.

In conclusion, salinity did not change the toxicity of the herbicide applied in low dose (1 nmol·dm^{-3}), but it did increase herbicide toxicity at high concentration (100 nmol·dm^{-3}) regarding the maize shoots.

KEY WORDS: sulfonylureas, rimsulfuron, salinity, maize, growth reduction, photosynthetic pigments, protein.

INTRODUCTION

Herbicides are used extensively in agriculture, they are efficient tools in reducing the weeds and improve the yields and quality of crops. Generally, action and metabolism of herbicides are well established, but there is little information on the influence of environmental conditions on action of herbicides (Mazur and Falco 1989). Thus, an important question arises, whether environmental factors can modify herbicides' phytotoxicity. A few researches indicate that environmental factors influence the efficiency of herbicides (Dickson et al. 1990; Sahid et al. 1996; Undabeytia et al. 1996). Soil salinity is an important factor limiting agricultural productivity in many parts of the world. Therefore, there is a need for more knowledge on interaction of herbicides with salinity.

Sulfonylurea herbicides represent a new generation of herbicides used at low rates (in grams per hectare) and show a very low toxicity to animals. These herbicides inhibit the activity of acetolactate synthase (ALS), the enzyme that catalyzes the first step in the biosynthesis of the branched amino acids – valine, leucine and isoleucine – and then alter protein metabolism (Ray 1984). It has been well documented that sulfonylureas reduce the availability of branched amino acid in treated plants, nevertheless the consequence of that reduction is an increase in the other amino acids (Royuela et al. 1991; Scarponi et al. 1995; Fayez and Kristen 1996). It is worthy of mention, that a low level of branched amino acids does not exert an inhibitory effect on protein accumulation in some plants (Clayton and Reynolds 1991).

The objective of this study was to determine the extent at which salinity modifies maize response to Titus 25 DF – sulfonylurea herbicide (with a. i. – active ingredient rimsulfuron – N-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide). Considering the role of salinity in Titus toxicity to maize, we examined the growth parameters (fresh and dry weight,

length) and some biochemical constituents, i.e. photosynthetic pigments, total protein and free amino compounds. All tested biochemical compounds are important components of stress-coping mechanisms (Lichtenthaler 1996). Generally, maize is tolerant to sulfonylurea herbicide, but there are differences in tolerance among their cultivars (Demczuk 1996). For our experiments we had chosen two doses of herbicide: subinhibitory concentration of 1 nmol·dm^{-3} a.i. and concentration of 100 nmol·dm^{-3} a.i. effectively applied to weed control.

MATERIAL AND METHODS

Maize (*Zea mays* L. var. Kometa, seeds were obtained from "Nasiona Kobierzyc" Company) after germination (48 h) was grown in beakers filled with the following media: modified Knop solution (control), Knop solution with the addition of 60 mmol·dm^{-3} NaCl (salt treatment), rimsulfuron at concentration of 1 or 100 nmol·dm^{-3} (herbicide treatments) and both 60 mmol·dm^{-3} NaCl and rimsulfuron (salt and herbicide treatments). Culture conditions were as follows: 16 h photoperiod (220 µmol·m^{-2}·s^{-1}) at 26/20°C day/night temperature, 65-70% relative humidity. After 7 days of cultivation in the nutrient solution plant growth was determined (shoot and root length, fresh and dry weight) as well as total protein content, free amino compounds and photosynthetic pigments.

The contents of protein and free amino acids were determined in dry plant material. Plants organs (shoots and roots) were dried for 2 h at 105°C and than for 48 h at 70°C. Plant samples (50 mg of whole roots or second and third leaf) were homogenized in 7 cm^{-3} of 5% trichloroacetic acid (TCA) and centrifuged 15 min at 18 000 g. After centrifugation the supernatant was adjusted to pH 5.5÷7.0 and amino compounds were determined using the ninhydrine reagent (Weber et al. 1991). The pellet obtained after first centrifugation was dissolved in 1 mol·dm^{-3} NaOH and stored for 10 min at 100°C. Than the aliquots were centrifuged (10 min at 18 000 g) and NaOH-soluble protein in supernatant was determined by the Bradford's method (1976) with bovine serum albumin (BSA) as the standard. The experiments were repeated six times.

Photosynthetic pigments were extracted from the second leaf of maize using 80% acetone and the concentrations of chlorophylls and carotenoids were calculated using Arnon equations (1949). The experiments were repeated three times.

All mean values were analysed statistically and the least significant difference (LSD, $p<0.05$) was used for means comparisons.

RESULTS

Plant growth (Fig. 1)

Salt stress and herbicide at concentration of 100 nmol·dm^{-3} led to significant inhibition in shoot and root growth of maize, but NaCl did not inhibit roots lengths. Rimsulfuron at concentration of 1 nmol·dm^{-3} did not significantly change the growth parameters of maize. Salt plus 1 nmol·dm^{-3} herbicide caused inhibition of plant growth, but that inhibition was due to the salt. NaCl plus 100 nmol·dm^{-3} herbicide led to the most significant decrease in maize growth. The shoots were less sensitive than roots to herbicide alone, as well as to herbicide plus NaCl. In the case of shoots the combined effect of NaCl and herbicide was additive. The reduction of shoot fresh weight reached 38% and 25% respectively for salt condition and in the presence of 100 nmol·dm^{-3} rimsulfuron, whereas under NaCl plus herbicide it amounted to 64%. The decrease in dry weight of maize shoots, grown in nutrient solution with the addition of NaCl plus 100 nmol·dm^{-3} herbicide was very high, but not as high as the reduction in fresh weight, which ranged up to 51%. Root growth was dramatically inhibited by the herbicide at concentration of 100 nmol·dm^{-3}, whereas NaCl caused a lower inhibition of root growth. In the presence of 100 nmol·dm^{-3} herbicide the root length amounted only to 31% of the control, and fresh and dry weight were 18% and 29% of the control respectively. Combined action of NaCl and 100 nmol·dm^{-3} herbicide caused the greatest inhibition of growth, but the differences between herbicide and NaCl plus herbicide treatments remained insignificant.

Protein and amino compound contents (Fig. 2A, 2B)

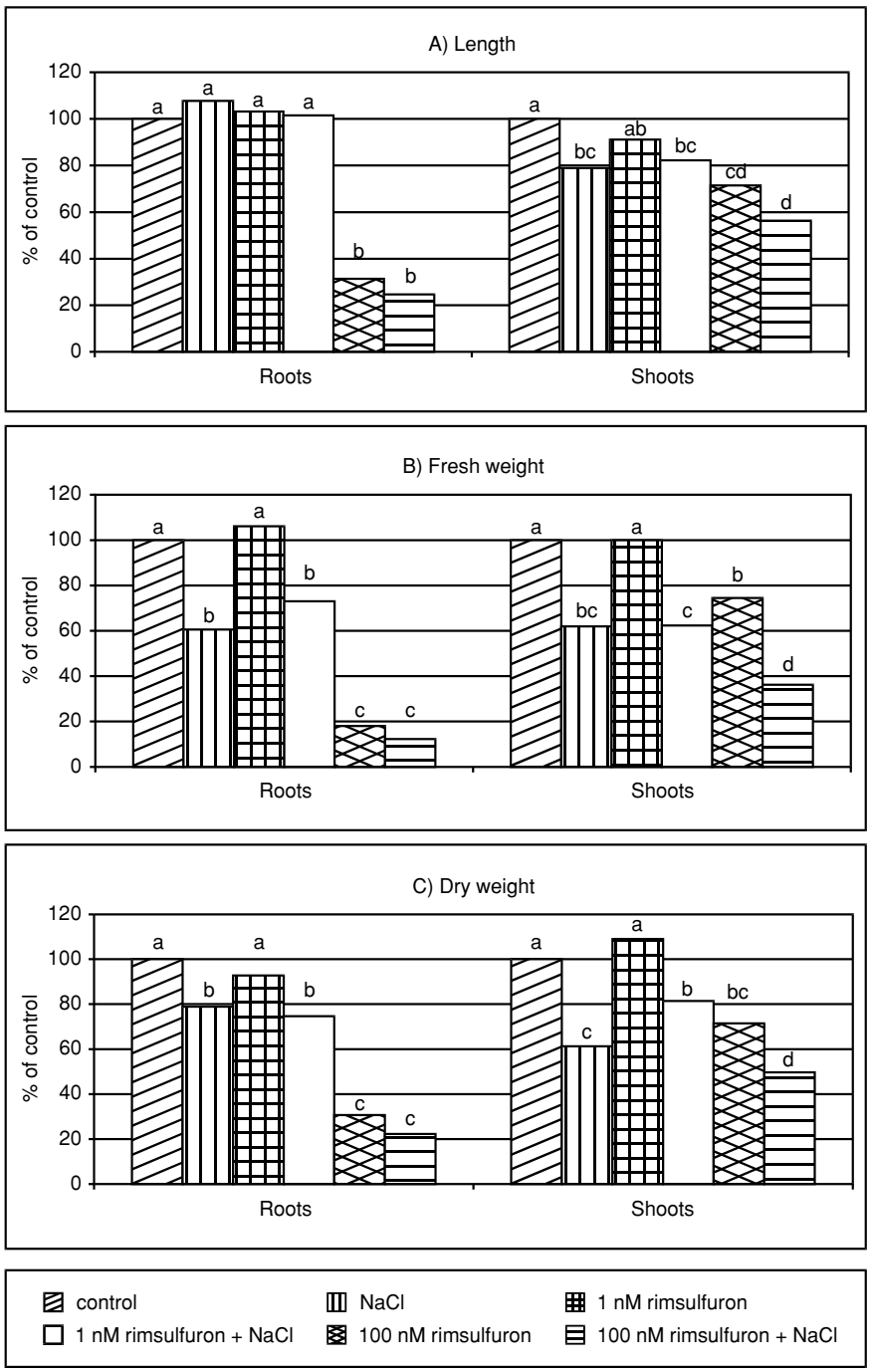
All stress factors led to the increase in total protein content and free amino compounds level in maize leaves. Both concentrations of herbicide and herbicide plus NaCl caused approximately a 20% increase in protein level, as compared to control plant. NaCl did not change significantly the protein content in leaves, but it caused a 2.8-fold increase in amino compounds. The remaining stress factors also augmented accumulation of amino compounds, but these changes were less significant than under NaCl stress. The concentration of total protein in maize roots decreased under NaCl and 100 nmol·dm^{-3} rimsulfuron, but it was not significantly altered under 1 nmol·dm^{-3} rimsulfuron and herbicide plus NaCl. All stress treatments caused a cosiderable increase in amino compounds level in maize roots, with the exception of 100 nmol·dm^{-3} rimsulfuron plus NaCl. The highest increase in free amino compounds was observed in the presence of 1 nmol·dm^{-3} rimsulfuron and 1 nmol·dm^{-3} rimsulfuron plus NaCl and it amounted approximately to 250% of the control.

Photosynthetic pigments (Fig. 2C)

The changes in total chlorophyll content were limited. However, carotenoid concentration was markedly reduced by all investigated stress factors. Nevertheless, the combined action of 100 nmol·dm^{-3} rimsulfuron and NaCl caused a 10% increase in carotenoid content, as compared to the control plants.

DISCUSSION

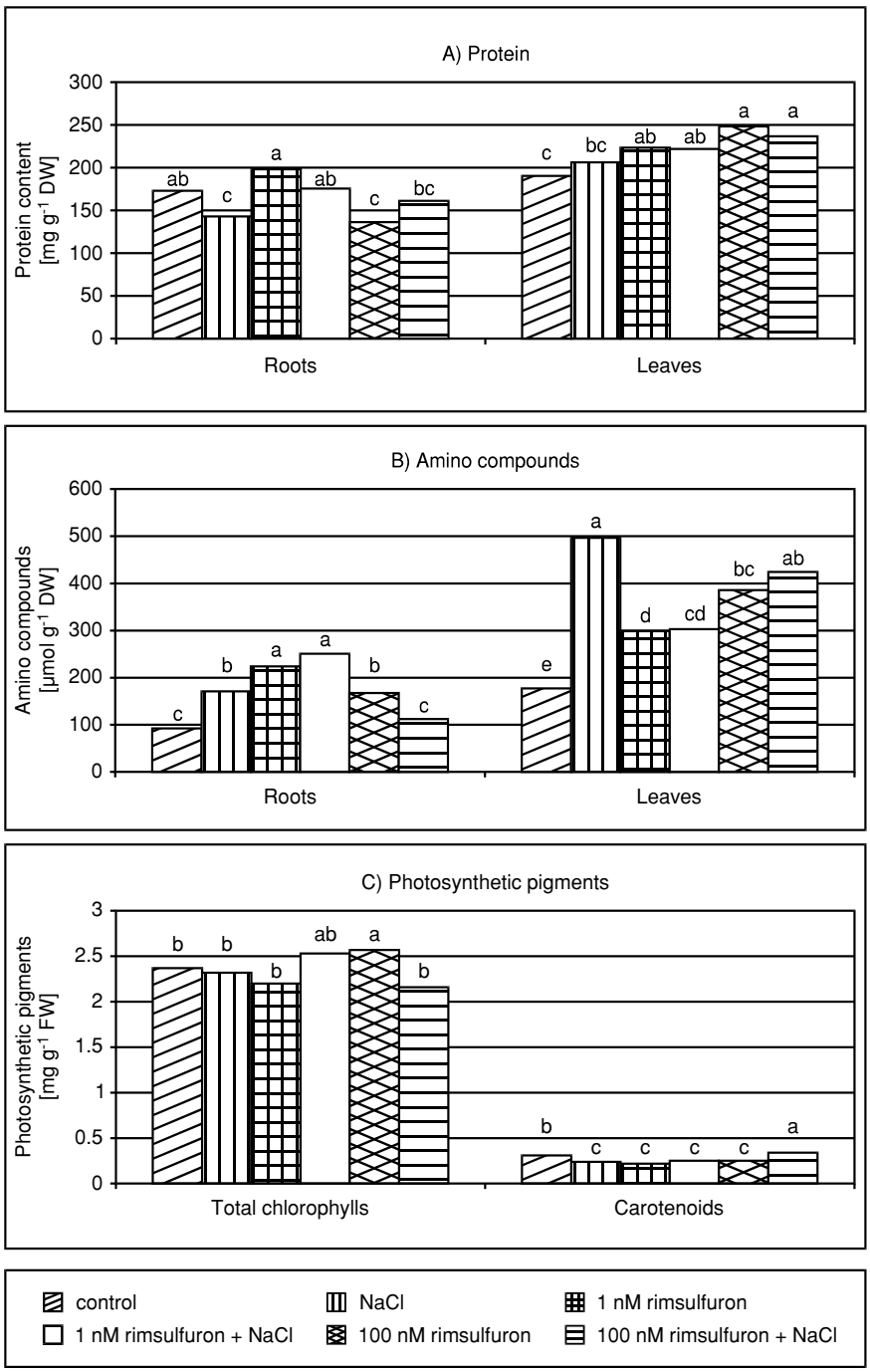
To determine the influence of salinity on rimsulfuron phytotoxicity, we analysed the growth parameters and some biochemical constituents such as total protein, free amino compounds and photosynthetic pigments. The growth response to sulfonylurea herbicides shows that maize var. Kometa is tolerant to rimsulfuron when applied at low concentration. In the presence of low concentration of rimsulfuron (1 nmol·dm^{-3}) plus NaCl there is no interaction between herbicide and salinity, and maize growth inhibition is due to NaCl. At high concentration of rimsulfuron



Differences between control and stress treatments were compared by LSD and values marked with the same letter do not differ significantly (5%).

Fig. 1. The influence of 60 mmol dm^{-3} NaCl, 1 and 100 nmol dm^{-3} rimsulfuron and NaCl plus rimsulfuron on length (A), fresh weight (B) and dry weight (C) of roots and shoots in maize after 7 days of treatment.

(100 nmol·dm^{-3}) the growth of maize is markedly reduced. Root growth is more reduced than shoot growth. This result is often observed if herbicides are applied to the nutrient solution (Royuela et al. 1991; Demczuk 1996; Sacała et al. 1999). Reduction in dry weight of shoot reached 29%, whereas the drop of root dry weight was 71%. Considerable reduction of root growth in the presence of sufonylurea herbicides was observed in many plants – maize, wheat, bean, pea, broad bean (Royuela et al. 1991; Fayez and Kristen 1996). If 100 nmol·dm^{-3} rimsulfuron was applied under salt conditions, the strongest inhibition in root and shoot growth was observed. The changes in length and fresh weight of shoots indicate that the combined effect of NaCl and rimsulfuron on maize is additive – the total response equals the sum of the particular factors. Reduction in fresh weight of shoot amounted to 38 and 25% in the presence of NaCl and rimsulfuron respectively. However, in the presence of NaCl plus rimsulfuron, it was 64%. In our previous study we showed that the inhibitory effect of NaCl plus glyphosate (a nonselective herbicide which inhibits aromatic amino acids biosynthesis) on shoot growth in maize was more significant than the inhibition due to each of the factor used separately (Sacała et al. 1999). Considering the combined action of 100 nmol·dm^{-3} rimsulfuron and NaCl on root growth, no similar correlations were found. In this case, the reduction of root growth was very high and



Differences between control and stress treatments were compared by LSD and values marked with the same letter do not differ significantly (5%).

Fig. 2. The influence of 60 mmol dm^{-3} NaCl, 1 and 100 nmol dm^{-3} rimsulfuron and NaCl plus rimsulfuron on total protein content (A), free amino compounds (B) and photosynthetic pigments (C) in maize after 7 days of treatment.

was caused by rimsulfuron. Rimsulfuron at 100 nmol·dm^{-3} concentration is very toxic to maize roots. Similar results were obtained for cucumber (Kuc et al. 2002). This is not surprising, because sulfonylurea herbicides drastically inhibit cell divisions in roots (Ray 1982; Rost 1984; Clayton and Reynolds 1991). Fayez and Kristen (1996) suggest that the herbicide-induced root growth reduction may be partially due to the injuries of root caps.

The data regarding biochemical constituents indicate that the investigated stress factors led to significant changes in total protein and free amino compound contents. Rimsulfuron applied at 1 and 100 nmol·dm^{-3} concentration caused a marked increase in protein and amino compound level in leaves and in amino compound contents in maize roots. However, 100 nmol·dm^{-3} rimsulfuron led to a decrease in root protein concentration. Rimsulfuron, like the other sulfonylurea herbicides, reduces the concentration of branched amino acids in the total amino acid pool. Nevertheless, there are reports indicating the increase in total free amino acid contents of plant treated with sulfonylureas (Royuela et al. 1991). Our data show that the most significant increase in free amino acid contents appeared in leaves of maize grown under NaCl stress. This high accumulation of free amino compounds may contribute to the osmoregulation and protection of proteins and membranes in conditions of salt stress (Delauney and Verma 1993; Ha-

segawa et al. 2000). Accumulation of soluble nitrogen compounds in response to environmental stresses seems to be a widespread phenomenon (Levy 1983; Delauney and Verma 1993; Costa and Morel 1994; Gzik 1996; Gilbert et al. 1998; Aziz et al. 1999; Hasegawa et al. 2000; Sacała et al. 2002). Among these compounds free amino acids play an important role. The analysis of total protein content indicates that accumulation of free amino compounds is not associated with protein degradation. An increase in total protein content in leaves may be due to synthesis and accumulation of "stress proteins" (Clayton and Reynolds 1991; Riccardi et al. 1998; Scott-Craig et al. 1998; Campalans et al. 1999; Hasegawa et al. 2000). In maize roots, a high concentration of free amino compounds was observed in the presence of 1 nmol·dm^{-3} rimsulfuron and 1 nmol·dm^{-3} rimsulfuron plus NaCl, whereas 100 nmol·dm^{-3} rimsulfuron plus NaCl had no effect. An increase in free amino compounds under 100 nmol·dm^{-3} rimsulfuron appears to be a consequence of protein proteolysis. The fall of total protein content under this condition supports that suggestion.

The total chlorophyll concentrations for all treatments did not change considerably, while the carotenoid content decreased significantly. These results support the conclusion that carotenoids are more sensitive to the investigated stressors than chlorophylls. It is possible that stress factors, which do not directly affect the synthesis of chlorophylls, reduce chlorophyll contents in the long run (Lichtenthaler 1996; Younis et al. 2000). The combined action of 100 nmol·dm^{-3} rimsulfuron and NaCl caused a significant increase in carotenoid content. That unexpected increase displayed an antagonistic effect of interaction between salinity and herbicide. The marked increase in carotenoid contents in the presence of NaCl plus herbicide was observed in maize and cucumber (Sacała et al. 1999; Kuc 2002). It is well known that carotenoids are an important component of the antioxidative system in plants (Burton and Ingold 1984; Siefermann-Harms 1987). This oxidative stress in plant cells may be due to many different abiotic factors (Scandalios 1993).

CONCLUSIONS

Our experiments were performed to determine the possible interaction between herbicide phytotoxicity and salinity. These interactions might be considered as an increase or a decrase in herbicide toxicity to plants. In our experiments the definite tendencies in the combined action of rimsulfuron and NaCl were not recorded. This study indicates that salinity does not change the toxicity of the herbicide applied in a low dose (1 nmol·dm^{-3}). A high dose of herbicide (100 nmol·dm^{-3}) was very toxic to maize roots and, under combined action of herbicide and NaCl, the inhibitory effect was caused by the herbicide itself. However, in the case of maize shoots, the negative effect of NaCl plus herbicide equalled the sum of negative effects exerted by each component applied separately (additive effect).

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REAKCJA KUKURYDZY (*ZEA MAYS* L.) NA RIMSULFURON W WARUNKACH ZASOLENIA

STRESZCZENIE

Badania prowadzono w celu stwierdzenia czy istnieją interakcje pomiędzy zasoleniem (60 mmol·dm^{-3} NaCl) a fitotoksycznością herbicydu Titus 25 DF (herbicyd sulfonylomocznikowy, substancja aktywna – rimsulfuron). Herbicyd stosowano w dwóch stężeniach (1 i 100 nmol·dm^{-3}) i dodawano bezpośrednio do pożywki. Po siedmiu dniach wzrostu roślin w pożywce określono parametry wzrostowe roślin oraz zawartość podstawowych składników takich jak: barwniki fotosyntetyczne, białko całkowite oraz wolne związki aminowe.

Równoczesne działanie 1 nmol·dm^{-3} rimsulfuronu i NaCl spowodowało redukcję biomasy roślin, jednak obniżka ta wynikała z obecności soli. Rimsulfuron o stężeniu 100 nmol·dm^{-3} spowodował drastyczne zahamowanie wzrostu korzeni, obecność soli nie miała większego wpływu. Natomiast w przypadku pędów kukurydzy zahamowanie ich wzrostu pod wpływem łącznego działania NaCl i 100 nmol·dm^{-3} rimsulfuronu było równe sumie procentowych zahamowań wzrostu spowodowanych pojedynczym czynnikiem, jest to więc oddziaływanie addytywne. Redukcja świeżej masy pędów kukurydzy wyniosła aż 64%.

Czynniki stresowe spowodowały duży wzrost zawartości związków aminowych w badanych tkankach, wyjątek stanowiły korzenie kukurydzy rosnącej w obecności NaCl plus 100 nmol·dm^{-3} rimsulfuron. Zawartość białka całkowitego w korzeniach kukurydzy obniżyła się w obecności NaCl oraz 100 nmol·dm^{-3} rimsulfuronu, zaś pod wpływem pozostałych czynników nie zmieniała się znacząco. Wszystkie czynniki, z wyjątkiem 60 mol·dm^{-3} NaCl, indukowały niewielki wzrost zawartości białka w liściach kukurydzy. Pod wpływem badanych czynników zawartość chlorofilu nie zmieniała się znacząco natomiast stężenie karotenoidów obniżało się. Jednak łączne działanie 100 nmol·dm^{-3} rimsulfuronu i NaCl spowodowało 10% wzrost zawartości karotenoidów.

Podsumowując można stwierdzić, że fitotoksyczność rimsulfuronu stosowanego w niskiej dawce (1 nmol·dm^{-3}) nie zmienia się w warunkach zasolenia natomiast wysokie stężenie (100 nmol·dm^{-3}) staje się bardziej toksyczne w stosunku do części nadziemnych kukurydzy.

SŁOWA KLUCZOWE: sulfonylomoczniki, rimsulfuron, zasolenie, kukurydza, zahamowanie wzrostu, barwniki fotosyntetyczne, białko.