

# **EFFECT OF MANGANESE DEFICIENCY ON GAS EXCHANGE PARAMETERS, LEAF GREENNESS (SPAD) AND YIELD OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.) AND ORCHARD GRASS (*DACTYLIS GLOMERATA* L.)**

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## Abstract

The objective of this study was to determine the effect of manganese deficiency in soil on the rate of photosynthesis and transpiration, water use efficiency, leaf greenness and the yield of selected cultivars of perennial ryegrass (*Lolium perenne* L.) and orchard grass (*Dactylis glomerata* L.). During the growing season, the rate of photosynthesis and transpiration was measured using a LI-COR 6400 gas analyzer (Portable Photosynthesis System), and leaf greenness was estimated with a Minolta SPAD-502 chlorophyll meter. Water use efficiency (WUE) was calculated based on instantaneous values of photosynthesis and transpiration. Dry matter yield was determined by green matter drying to constant weight at 105°C. The results of the study indicate that the response of grasses to manganese deficiency in soil was dependent on plant species and cultivar. In the present experiment perennial ryegrass cultivars showed a stronger response to manganese deficit than orchard grass cultivars. Their response involved a decrease in the rate of photosynthesis and transpiration, and in the chlorophyll content of leaves. Among the tested cultivars, perennial ryegrass cv. Maja was found to be most sensitive to manganese deficiency in soil, as confirmed by the highest decrease in the values of all examined parameters.

**Key words:** manganese deficiency, perennial ryegrass, orchard grass, photosynthesis, transpiration, water use efficiency (WUE), leaf greenness (SPAD), yield.

## WPLYW NIEDOBORU MANGANU NA WSKA•NIKI WYMIANY GAZOWEJ, INDEKS ZIELONOŚCI LIŚCI (SPAD) ORAZ PLONOWANIE ŻYCICY TRWAŁEJ (*LOLIUM PERENNE* L.) I KUPKÓWKI POSPOLITEJ (*DACTYLIS GLOMERATA* L.)

### Abstrakt

Celem pracy była ocena wpływu niedoboru manganu w glebie na intensywność fotosyntezy i transpiracji, współczynnik wykorzystania wody, indeks zieloności liści oraz plonowanie wybranych odmian życicy trwałej (*Lolium perenne* L.) i kupkówki pospolitej (*Dactylis glomerata* L.). W okresie wegetacji mierzono intensywność fotosyntezy i transpiracji za pomocą przenośnego analizatora gazowego LI-COR 6400 oraz indeks zieloności liści za pomocą optycznego chlorofilometru Minolta SPAD-502. Na podstawie chwilowych wartości fotosyntezy i transpiracji wyliczono współczynnik wykorzystania wody (WUE). Plon suchej masy określono przez wysuszenie zielonej masy w temperaturze 105°C, do stałej wagi. Wykazano, że reakcja traw na niedobór manganu w glebie zależy od gatunku rośliny i odmiany. Większą reakcję na deficyt manganu, polegającą na ograniczeniu procesu fotosyntezy i transpiracji oraz zmniejszeniu zawartości chlorofilu w liściach, wykazywały odmiany życicy trwałej niż kupkówki pospolitej. Najbardziej wrażliwa na niedobór manganu w glebie była odmiana życicy trwałej Maja, u której stwierdzono w największym stopniu ograniczenie wszystkich badanych parametrów.

Słowa kluczowe: niedobór manganu, życica trwała, kupkówka pospolita, fotosynteza, transpiracja, WUE, indeks SPAD, plonowanie.

## INTRODUCTION

Manganese is essential for the growth and development of plants (JANKOWSKI et al. 2000, CIOROI, FLOREA 2003). This element is a growth stimulator and an activator of numerous enzymatic processes. It also participates in nitrogen assimilation, the synthesis of proteins and vitamin C, respiration, as well as in the reactions of splitting water to liberate oxygen during photosynthesis (GRZYŚ 2004). Moreover, manganese affects chlorophyll persistence, and its deficiency causes chlorophyll breakdown under the influence of strong light. Manganese deficit disturbs normal growth and development of plants. The symptoms of manganese deficiency in grasses include leaf chlorosis, a slower growth rate and failure to respond to nitrogen and iron (FALKOWSKI et al. 1990). Manganese deficiency occurs most often in crops grown on neutral carbonate soils and on soils with a high humus content (KABATA-PENDIAS, PENDIAS 1999, JODEŁKA et al. 2000, HALASOVA et al. 2001, KOPITKE, MENZIES 2004).

The objective of this study was to determine the effect of manganese deficiency in soil on the rate of photosynthesis and transpiration, water use efficiency, leaf greenness and the yield of selected cultivars of perennial ryegrass and orchard grass.

## MATERIALS AND METHODS

The experiment was conducted in a greenhouse of the University of Warmia and Mazury in Olsztyn. Kick-Brauckmann pots were filled with 10 kg of soil developed from loose sand, characterized by a low content of phosphorus ( $31 \text{ mg} \cdot \text{kg}^{-1}$ ), potassium ( $42 \text{ mg} \cdot \text{kg}^{-1}$ ), magnesium ( $13 \text{ mg} \cdot \text{kg}^{-1}$ ) and manganese ( $8.7 \text{ mg} \cdot \text{kg}^{-1}$ ). Soil reaction was slightly acidic – pH 5.8  $\text{dm}^{-3}$  in 1 mol KCl. The experiment was performed in four replications. In each pot, 2 to 3 seeds of grass were sown at 10 points, and the plants were thinned immediately after emergence, leaving 7 plants per pot. The experiment involved two cultivars of perennial ryegrass (*Lolium perenne* L.): tetraploid Maja and diploid Argona, and two cultivars of orchard grass (*Dactylis glomerata* L.): tetraploid Dala and diploid Areda. Control pots were fertilized with a nutrient solution containing 1.00 g N as  $\text{CO}(\text{NH}_2)_2$ , 0.25 g P as  $\text{KH}_2\text{PO}_4$ , 1.00 g K as  $\text{K}_2\text{SO}_4$  and 0.50 g Mg as  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . A micronutrient solution ( $30 \text{ ml} \cdot \text{pot}^{-1}$ ), composed of 2.65 g Fe in EDTA, 0.09 g  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.1 g  $\text{ZnCl}_2$ , 0.03 g  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.12 g  $\text{H}_3\text{BO}_3$  and 0.01 g  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  per kg of soil, was also applied. The remaining pots were not fertilized with manganese. During the growing season leaf greenness was estimated with a Minolta SPAD-502 chlorophyll meter, while the rate of photosynthesis and transpiration was measured using a LI-COR 6400 gas analyzer (Portable Photosynthesis System), at air temperature of around  $25^\circ\text{C}$ , a constant  $\text{CO}_2$  concentration of 400 ppm and illumination of  $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Four measurements were performed for each regrowth. Mean values for regrowths are presented in the paper. Water use efficiency (WUE) was calculated based on instantaneous values of photosynthesis and transpiration. The aboveground parts of plants were cut three times over the growing season. Dry matter yield was determined by green matter drying to constant weight at  $105^\circ\text{C}$ . The results were processed statistically with the use of Statistica software. The significance of differences was verified by Tukey's test at  $p = 0.99$ .

## RESULTS AND DISCUSSION

The tested cultivars showed low intensity of photosynthesis, ranging from 5.58 to  $8.88 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$  on average (Table 1). Cv. Argona was marked by the highest rate of photosynthesis. Manganese deficiency in soil significantly limited photosynthesis intensity in all tested cultivars. The present results are consistent with the findings of other authors (OHKI 1985, SIENKIEWICZ-CHOLEWA 2002) who also observed a lower rate of photosynthesis in manganese-deficient treatments. In the present experiment the rate of photosynthesis decreased by 23% under manganese deficit conditions, com-

Table 1

Intensity of photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )					
Cultivars	Fertilization	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	Mean
Areda	control object manganese deficiency	5.98 <sup>bc</sup>	9.17 <sup>d</sup>	7.38 <sup>e</sup>	7.51 <sup>d</sup>
		6.49 <sup>bc</sup>	7.43 <sup>a</sup>	4.62 <sup>a</sup>	6.18 <sup>b</sup>
Dala	control object manganese deficiency	7.16 <sup>c</sup>	8.39 <sup>bc</sup>	5.92 <sup>c</sup>	7.16 <sup>cd</sup>
		5.55 <sup>b</sup>	8.69 <sup>cd</sup>	6.59 <sup>d</sup>	6.95 <sup>c</sup>
Argona	control object manganese deficiency	9.46 <sup>d</sup>	10.25 <sup>e</sup>	6.93 <sup>d</sup>	8.88 <sup>e</sup>
		5.81 <sup>b</sup>	8.00 <sup>b</sup>	4.51 <sup>a</sup>	6.11 <sup>b</sup>
Maja	control object manganese deficiency	6.30 <sup>bc</sup>	10.43 <sup>e</sup>	9.00 <sup>f</sup>	8.58 <sup>e</sup>
		3.07 <sup>a</sup>	8.13 <sup>b</sup>	5.53 <sup>b</sup>	5.58 <sup>a</sup>
Mean for cultivars					
Areda		6.23 <sup>b</sup>	8.30 <sup>a</sup>	6.00 <sup>b</sup>	6.84 <sup>a</sup>
Dala		6.36 <sup>b</sup>	8.54 <sup>a</sup>	6.26 <sup>c</sup>	7.05 <sup>a</sup>
Argona		7.63 <sup>c</sup>	9.13 <sup>b</sup>	5.72 <sup>a</sup>	7.49 <sup>b</sup>
Maja		4.68 <sup>a</sup>	9.28 <sup>b</sup>	7.28 <sup>d</sup>	7.08 <sup>a</sup>
Mean for fertilization					
Control object		7.22 <sup>b</sup>	9.56 <sup>b</sup>	7.31 <sup>b</sup>	8.03 <sup>b</sup>
Manganese deficiency		5.23 <sup>a</sup>	8.06 <sup>a</sup>	5.31 <sup>a</sup>	6.20 <sup>a</sup>
Mean for cuts		6.23 <sup>a</sup>	8.81 <sup>b</sup>	6.31 <sup>a</sup>	7.12 <sup>a</sup>

\*homogeneous statistical groups (values marked with same letter did not differ statistically)

pared to control treatments. The response of perennial ryegrass cultivars to manganese deficiency was stronger than that of orchard grass cultivars, as confirmed by a higher decrease in photosynthesis intensity. This could be due to the ability of orchard grass to accumulate and store large quantities of manganese (DOBOSZYŃSKI, WASILEWSKI, 1983, MŁYNARCZYK, OLKOWSKI 1986, JARGIELLO et al. 1991). Cv. Maja was found to be least resistant to manganese deficiency. The rate of photosynthesis decreased by 35% in this cultivar. In both species and in all cultivars the highest rate of  $\text{CO}_2$  assimilation was recorded in the second regrowth.

Manganese deficiency limited water transpiration per unit leaf area. The value of this indicator was on average 24% lower in manganese-deficient treatments, in comparison with control treatments (Table 2). Orchard grass responded only slightly to manganese deficiency – the rate of transpiration in the tested cultivars of this species did not differ significantly subject to the level of manganese fertilization. On the other hand, the value of this parameter decreased by 34% and 42% in perennial ryegrass cultivars not fertilized with manganese. The highest rate of transpiration and the highest reduction in water evaporation under manganese deficit conditions were noted in cv. Maja. These results contradict the findings of OHKI (1985) who postulated that manganese deficiency had no effect on transpiration in common wheat. It should be also stressed that particular regrowths differed with respect to the rate of transpiration per unit leaf area. The highest rate

Table 2

Intensity of transpiration ( $\text{m mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )					
Cultivars	Fertilization	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	Mean
Areda	control	0.90 <sup>a</sup>	0.92 <sup>d</sup>	0.52 <sup>c</sup>	0.78 <sup>c</sup>
	manganese deficiency	1.32 <sup>d</sup>	0.68 <sup>a</sup>	0.28 <sup>a</sup>	0.76 <sup>c</sup>
Dala	control	1.04 <sup>b</sup>	0.77 <sup>b</sup>	0.53 <sup>c</sup>	0.78 <sup>c</sup>
	manganese deficiency	1.05 <sup>b</sup>	0.80 <sup>c</sup>	0.51 <sup>c</sup>	0.79 <sup>c</sup>
Argona	control	1.21 <sup>c</sup>	1.31 <sup>f</sup>	0.38 <sup>b</sup>	0.96 <sup>d</sup>
	manganese deficiency	0.81 <sup>a</sup>	0.81 <sup>c</sup>	0.28 <sup>a</sup>	0.64 <sup>a</sup>
Maja	control	2.03 <sup>e</sup>	1.19 <sup>e</sup>	0.36 <sup>b</sup>	1.19 <sup>e</sup>
	manganese deficiency	1.05 <sup>b</sup>	0.76 <sup>b</sup>	0.26 <sup>a</sup>	0.69 <sup>b</sup>
Mean for cultivars					
Areda		1.11 <sup>b</sup>	0.80 <sup>a</sup>	0.40 <sup>c</sup>	0.77 <sup>a</sup>
Dala		1.05 <sup>a</sup>	0.78 <sup>a</sup>	0.52 <sup>d</sup>	0.78 <sup>a</sup>
Argona		1.01 <sup>a</sup>	1.06 <sup>c</sup>	0.33 <sup>b</sup>	0.80 <sup>b</sup>
Maja		1.54 <sup>c</sup>	0.97 <sup>b</sup>	0.31 <sup>a</sup>	0.94 <sup>c</sup>
Mean for fertilization					
Control object		1.29 <sup>b</sup>	1.04 <sup>b</sup>	0.45 <sup>b</sup>	0.93 <sup>b</sup>
Manganese deficiency		1.06 <sup>a</sup>	0.76 <sup>a</sup>	0.33 <sup>a</sup>	0.71 <sup>a</sup>
Mean for cuts		1.18 <sup>c</sup>	0.90 <sup>b</sup>	0.39 <sup>a</sup>	0.82 <sup>b</sup>

Explanations, see Table 1

of transpiration was recorded in the first regrowth, and then it gradually decreased.

Manganese deficiency in soil had no considerable influence on water relations in the investigated species and cultivars of grass. In the cultivars Argona and Maja water use efficiency decreased only slightly, by 3% and 5% respectively, while in the cultivars of orchard grass WUE increased to a slight degree (Table 3). A comparison of water use efficiency in the tested cultivars revealed that it reached the highest level in cv. Maja. In all cultivars the highest water use efficiency was observed in the third regrowth, which resulted from a low rate of transpiration.

Leaf greenness measurements showed that orchard grass cultivars contained less chlorophyll in leaves than perennial ryegrass cultivars (Table 4). Among the cultivars of perennial ryegrass, cv. Maja had a significantly higher chlorophyll content (44.83 SPAD units on average). Manganese deficit resulted in a decrease in chlorophyll concentrations, by 5.5% on average. Cv. Maja showed the strongest response to manganese deficiency, although the chlorophyll content of leaves decreased in this cultivar by only 8% on average. Literature on the subject provides no information on the impact of manganese fertilization on the chlorophyll content of grasses, which makes a detailed discussion of the results impossible. However, in a study conduct-

Table 3

Water use efficiency ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \cdot \text{m mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )

Cultivars	Fertilization	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	Mean
Areda	control object manganese deficiency	6.70 <sup>c</sup>	10.02 <sup>b</sup>	14.26 <sup>bc</sup>	10.33 <sup>ab</sup>
		4.93 <sup>b</sup>	10.96 <sup>c</sup>	16.64 <sup>de</sup>	10.84 <sup>bc</sup>
Dala	control object manganese deficiency	6.89 <sup>c</sup>	10.93 <sup>c</sup>	11.28 <sup>a</sup>	9.70 <sup>a</sup>
		5.29 <sup>b</sup>	10.90 <sup>c</sup>	12.93 <sup>ab</sup>	9.71 <sup>a</sup>
Argona	control object manganese deficiency	7.85 <sup>c</sup>	7.84 <sup>a</sup>	18.38 <sup>e</sup>	11.36 <sup>c</sup>
		7.16 <sup>c</sup>	9.88 <sup>b</sup>	15.99 <sup>cd</sup>	11.01 <sup>bc</sup>
Maja	control object manganese deficiency	3.10 <sup>a</sup>	8.78 <sup>b</sup>	24.86 <sup>g</sup>	12.25 <sup>d</sup>
		2.93 <sup>a</sup>	10.78 <sup>c</sup>	21.07 <sup>f</sup>	11.56 <sup>cd</sup>
Mean for cultivars					
Areda		5.82 <sup>b</sup>	10.49 <sup>c</sup>	15.45 <sup>b</sup>	10.58 <sup>b</sup>
Dala		6.09 <sup>b</sup>	10.91 <sup>d</sup>	12.11 <sup>a</sup>	9.70 <sup>a</sup>
Argona		7.50 <sup>c</sup>	8.86 <sup>a</sup>	17.18 <sup>c</sup>	11.18 <sup>c</sup>
Maja		3.01 <sup>a</sup>	9.78 <sup>b</sup>	22.96 <sup>d</sup>	11.92 <sup>d</sup>
Mean for fertilization					
Control object Manganese deficiency		6.13 <sup>b</sup>	9.39 <sup>a</sup>	17.19 <sup>a</sup>	10.91 <sup>a</sup>
		5.08 <sup>a</sup>	10.63 <sup>b</sup>	16.66 <sup>a</sup>	10.79 <sup>a</sup>
Mean for cuts		5.60 <sup>a</sup>	10.01 <sup>b</sup>	16.93 <sup>c</sup>	10.85 <sup>b</sup>

Explanations, see Table 1

Table 4

Leaf greenness index (SPAD)

Cultivars	Fertilization	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	Mean
Areda	control object manganese deficiency	36.13 <sup>bc</sup>	42.03 <sup>c</sup>	40.85 <sup>c</sup>	39.67 <sup>b</sup>
		34.45 <sup>a</sup>	40.05 <sup>b</sup>	38.38 <sup>b</sup>	37.63 <sup>a</sup>
Dala	control object manganese deficiency	35.43 <sup>ab</sup>	40.05 <sup>b</sup>	41.03 <sup>c</sup>	38.84 <sup>b</sup>
		36.43 <sup>bc</sup>	38.55 <sup>a</sup>	36.48 <sup>a</sup>	37.15 <sup>a</sup>
Argona	control object manganese deficiency	39.95 <sup>d</sup>	43.18 <sup>c</sup>	41.58 <sup>c</sup>	41.57 <sup>c</sup>
		37.80 <sup>c</sup>	40.43 <sup>b</sup>	41.30 <sup>bc</sup>	39.84 <sup>b</sup>
Maja	control object manganese deficiency	44.98 <sup>e</sup>	48.10 <sup>e</sup>	46.90 <sup>e</sup>	46.66 <sup>e</sup>
		40.45 <sup>de</sup>	45.85 <sup>d</sup>	42.60 <sup>d</sup>	43.00 <sup>d</sup>
Mean for cultivars					
Areda		35.29 <sup>a</sup>	41.04 <sup>b</sup>	39.61 <sup>a</sup>	38.64 <sup>a</sup>
Dala		35.93 <sup>a</sup>	39.30 <sup>a</sup>	38.75 <sup>a</sup>	37.99 <sup>a</sup>
Argona		38.88 <sup>b</sup>	41.80 <sup>b</sup>	41.44 <sup>b</sup>	40.70 <sup>b</sup>
Maja		42.72 <sup>c</sup>	46.98 <sup>c</sup>	44.75 <sup>c</sup>	44.83 <sup>c</sup>
Mean for fertilization					
Control object Manganese deficiency		39.12 <sup>b</sup>	43.34 <sup>b</sup>	42.59 <sup>b</sup>	41.69 <sup>b</sup>
		37.28 <sup>a</sup>	41.22 <sup>a</sup>	39.69 <sup>a</sup>	39.40 <sup>a</sup>
Mean for cuts		37.83 <sup>a</sup>	42.05 <sup>b</sup>	41.62 <sup>b</sup>	40.50 <sup>b</sup>

Explanations, see Table 1

Table 5

Dry matter yield (g · pot <sup>-1</sup> )					
Cultivars	Fertilization	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	Mean
Areda	control	7.43 <i>bc</i>	7.63 <i>a</i>	5.45 <i>ab</i>	20.50 <i>b</i>
	manganese deficiency	6.80 <i>a</i>	6.78 <i>a</i>	4.53 <i>a</i>	18.10 <i>a</i>
Dala	control	8.15 <i>bc</i>	7.43 <i>a</i>	5.68 <i>bc</i>	21.25 <i>bc</i>
	manganese deficiency	8.18 <i>bc</i>	7.08 <i>a</i>	5.78 <i>bc</i>	21.03 <i>bc</i>
Argona	control	7.50 <i>bc</i>	7.13 <i>a</i>	6.45 <i>bc</i>	21.08 <i>bc</i>
	manganese deficiency	8.05 <i>bc</i>	6.50 <i>a</i>	6.10 <i>bc</i>	20.65 <i>bc</i>
Maja	control	8.73 <i>c</i>	7.05 <i>a</i>	6.70 <i>c</i>	22.48 <i>c</i>
	manganese deficiency	6.48 <i>ab</i>	6.63 <i>a</i>	6.25 <i>bc</i>	19.36 <i>b</i>
Mean for cultivars					
Areda		7.11 <i>a</i>	7.20 <i>a</i>	4.99 <i>a</i>	19.30 <i>a</i>
Dala		8.16 <i>b</i>	7.25 <i>a</i>	5.73 <i>b</i>	21.14 <i>b</i>
Argona		7.78 <i>ab</i>	6.81 <i>a</i>	6.28 <i>bc</i>	20.86 <i>b</i>
Maja		7.61 <i>ab</i>	6.84 <i>a</i>	6.48 <i>c</i>	20.93 <i>b</i>
Mean for fertilization					
Control object		7.95 <i>b</i>	7.31 <i>b</i>	6.07 <i>b</i>	21.33 <i>b</i>
Manganese deficiency		7.38 <i>a</i>	6.75 <i>a</i>	5.66 <i>a</i>	19.79 <i>a</i>
Mean for cuts		7.71 <i>b</i>	7.06 <i>b</i>	5.87 <i>a</i>	20.64 <i>c</i>

Explanations, see Table 1

ed by OHKI (1985) manganese deficit in soil reduced chlorophyll concentrations in the leaves of common wheat, similarly as in the tested grass species. In the current experiment the lowest chlorophyll content of leaves was recorded in the first regrowth, regardless of a cultivar.

The yield of orchard grass cv. Areda was found to be the lowest, while the yields of the other investigated cultivars remained at a comparable level (Table 5). A lower yield of this cultivar was also noted in our earlier studies (OLSZEWSKA 2004, 2005, 2006). Manganese deficiency decreased the yield of grasses, by around 7% on average. The greatest effect of manganese deficit on yielding was observed in perennial ryegrass cv. Maja, whose yield declined by approximately 14% in comparison with control treatments. It should be stressed that this cultivar showed the strongest response to deficit in soil Mn, reflected by the highest drop in the values of all examined parameters.

## CONCLUSIONS

1. The response of grasses to manganese deficiency in soil was dependent on plant species and cultivar. Perennial ryegrass cultivars showed a stronger response to manganese deficit than orchard grass cultivars. Their

response involved a decrease in the rate of photosynthesis and transpiration, and in the chlorophyll content of leaves.

2. Among the tested cultivars, perennial ryegrass cv. Maja was found to be most sensitive to manganese deficiency in soil, as confirmed by the highest decrease in the values of all examined parameters.

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