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EFFECT OF COPPER 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

Copper is essential for the growth and development of plants. This micronutrient affects chlorophyll content, photosynthesis process and water relations in the plant. The objective of this study was to determine the effect of copper 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 copper deficiency significantly decreased the rate of photosynthesis and transpiration, chlorophyll concentration in leaves and the yield of all investigated cultivars. Perennial ryegrass cv. Maja was found to be most resistant to copper deficiency – it was characterized by a high rate of photosynthesis and transpiration, and by the highest chlorophyll content. The yield of cv. Maja attained under copper deficit conditions was comparable to that of other cultivars grown under control conditions.

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

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WPŁYW NIEDOBORU MIEDZI NA WSKAŹNIKI WYMIANY GAZOWEJ, INDEKS ZIELONOŚCI LIŚCI (SPAD) ORAZ PLONOWANIE ŻYCICY TRWAŁEJ (LOLIUM PERENNE L). I KUPKÓWKI POSPOLITEJ (*DACTYLIS GLOMERATA* L.)

Abstrakt

Miedź jest mikroelementem niezbędnym do prawidłowego wzrostu i rozwoju roślin. Wpływa na zawartość chlorofilu, proces fotosyntezy oraz gospodarkę wodną roślin. Celem pracy była ocena wpływu niedoboru miedzi 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 i 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 niedobór miedzi istotnie ograniczył intensywność fotosyntezy, transpiracji, poziom chlorofilu w liściach oraz plonowanie wszystkich badanych odmian. Odmianą najbardziej odporną na niedobór miedzi była Maja, która odznaczała się wysoką intensywnością fotosyntezy i transpiracji oraz największą zawartością chlorofilu. W warunkach niedoboru miedzi plonowała ona na poziomie pozostałych odmian uprawianych w warunkach kontrolnych.

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

INTRODUCTION

Copper is essential for the growth and development of plants. This micronutrient influences chlorophyll content, participates in the processes of protein and carbohydrate synthesis, and positively affects the activity of nitrate reductase (FALKOWSKI et al. 1990, KUDUK 1996, TERELAK et al. 1998, KABATA-PENDIAS, PENDIAS 1999, BARCZAK et al. 2006). Moreover, copper has an impact on the photosynthesis process and water relations in the plant. Copper deficiency decreases the rate of photosynthesis and increases the rate of respiration (RUSZKOWSKA, WOJCIESKA-WYSKUPAJTYS 1996), and it induces changes in the structure of leaf epidermis and stem tissue. Under copper deficit conditions, stomata, parenchymal and sclerenchymal cells of the stem become smaller, while xylem cells undergo deformations. These undesirable changes limit water flow in the soil-plant system, leading to plant wilting (DYKI, BORKOWSKI 2000). The copper content of plants varies widely depending on the part of plant, growth stage, species and variety, as well as on copper concentrations in soil and climate conditions (KABATA-PENDIAS 1996, RUSZKOWSKA et al. 1996a).

The objective of this study was to determine the effect of copper 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 $mg \cdot kg^{-1}$), potassium (42 $mg \cdot kg^{-1}$), magnesium (13 $mg \cdot kg^{-1}$) and copper (4.5 $mg \cdot kg^{-1}$). Soil reaction was slightly acidic - pH 5.8 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 $CO(NH_2)_2$, 0.25 g P as KH_2PO_4, 1.00 g K as K_2SO_4 and 0.50 g Mg as $MgSO_4 \cdot 7H_2O$. A micronutrient solution (30 $mg \cdot pot^{-1}$), composed of 2.65 mg Fe in EDTA, 0.09 mg $MnCl_2 \cdot 4H_2O$, 0.1 mg $ZnCl_2$, 0.03 mg $CuCl_2 \cdot 2H_2O$, 0.12 mg H_3BO_3 and 0.01 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ per kg of soil, was also applied. The remaining pots were not fertilized with copper. P, K and Mg were applied pre-sowing at a single rate, while nitrogen and microelements were applied at three rates: pre-sowing, after the first and second cutting. 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° C, a constant CO_2 concentration of 400 ppm, illumination of 1000 µmol m^{-2} s^{-1} and soil moisture content of around 70% of field water capacity. Measurements were taken on the youngest, fully developed leaf of shoots selected randomly in each treatment. Four measurements were performed for each regrowth, and readouts were taken at one-week intervals. 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: cut I – 9 July, cut II – 30 August, cut III – 25 October. Dry matter yield was determined by green matter drying to constant weight at 105°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

Copper deficiency disturbs the conversion of light energy into chemical energy, thus reducing the rate of photosynthesis and photosynthetic activity in plants. The average rate of photosynthesis was low in the leaves of all investigated grass cultivars, and it ranged from 5.59 to 8.88 μmol CO_2 $m^{-2}s^{-1}$ (Table 1). Cv. Maja was marked by the highest rate of photosynthesis. The course of this process was widely differentiated in particular regrowths. The lowest rate of photosynthesis was observed in the first regrowth, while the highest – in the second regrowth. Copper deficiency in soil significantly limited photosynthesis intensity in the leaves of all tested cultivars. Compared to control treatments, the rate of photosynthesis decreased by 25%. Tetraploid cultivars were more resistant to copper deficiency than diploid cultivars. Among the tested cultivars, cv. Dala was found to be most resistant to copper deficiency. The rate of photosynthesis decreased to the slightest degree in this cultivar, i.e. by 15% on average, compared to 22-36% in the other cultivars. Orchard grass is known for its ability to accumulate copper. FALKOWSKI et al. (1990) reported that the concentration of this micronutrient was substantially higher in orchard grass than in perennial ryegrass under copper deficit conditions. This probably explains the weaker response of orchard grass to copper deficiency, in comparison with perennial ryegrass.

In the present study copper deficiency decreased transpiration intensity, on average by 31% (Table 2). The investigated cultivars differed in their responses to copper deficit. The weakest response was recorded in cv. Maja, while the strongest in cv. Argona – the rate of transpiration decreased in these cultivars by approximately 16% and 42% respectively. DYKA and BORKOWSKI (2000) demonstrated that in tomato plants copper deficiency caused a reduction in the thickness of the xylem layer and deformation of xylem cells as well as the closing of stomata, thus disturbing water transport and limiting transpiration. Perennial ryegrass cultivars evaporated more water than orchard grass cultivars. Particular regrowths differed with respect to the rate of transpiration, which was the fastest in the first regrowth and the slowest in the third regrowth.

Water relations in the plant can be determined based on water use efficiency (WUE). The highest water use efficiency was observed in the third regrowth, which resulted from a low rate of transpiration (Table 3). In orchard grass cultivars, higher values of WUE were recorded in treatments not fertilized with copper, whereas in perennial grass cultivars – in control treatments. Among the tested cultivars, the lowest WUE (mean value for regrowths) was reported for cv. Argona, while differences between the remaining cultivars were statistically non-significant.

Copper participates in chlorophyll biosynthesis (GRZYŚ 2004) – around 70% of copper can be found in chloroplasts. Copper deficiency in soil had a signifi-

Table 1

Intensity of photosynthesis (μmol CO_2 m^{-2} s^{-1})

Cultivars	Fertilization	1st cut	2nd cut	3rd cut	Mean
Areda	control object copper deficiency	5.98*cd 3.36 a	9.17 c 8.29 b	7.38 d 5.12 a	7.51 e 5.59 a
Dala	control object copper deficiency	7.16 e 6.22 d	8.39 b 6.98 a	5.92 b 5.02 a	7.16 d 6.07 b
Argona	control object copper deficiency	9.46 f 4.36 b	10.25 d 6.82 a	6.93 c 5.87 b	8.88 f 5.69 a
Maja	control object copper deficiency	6.30 d 5.13 bc	10.43 d 8.96 c	9.01 e 5.91 b	8.58 f 6.66 c
Mean for cultivars					
Areda Dala Argona Maja		4.67 a 6.69 c 6.91 c 5.71 b	8.73 b 7.68 a 8.54 b 9.69 c	6.25 b 5.47 a 6.40 b 7.46 c	6.55 a 6.61 a 7.28 b 7.62 c
Mean for fertilization					
Control object Copper deficiency		7.22 b 4.77 a	9.56 b 7.76 a	7.31 b 5.48 a	8.03 b 6.00 a
Mean for cuts		5.60 a	8.66 b	6.39 a	7.02 a

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

Table 2

Intensity of transpiration (m mol H_2O m^{-2} s^{-1})

Cultivars	Fertilization	1st cut	2nd cut	3rd cut	Mean
Areda	control object copper deficiency	0.90 b 0.64 a	0.92 e 0.52 b	0.52 c 0.35 a	0.78 d 0.50 a
Dala	control object copper deficiency	1.04 c 0.92 b	0.77 c 0.44 a	0.53 c 0.36 a	0.78 d 0.57 b
Argona	control object copper deficiency	2.03 e 0.87 b	1.19 f 0.84 d	0.36 a 0.37 a	1.19 f 0.69 c
Maja	control object copper deficiency	1.21 d 1.10 c	1.31 g 0.84 d	0.38 a 0.48 b	0.96 e 0.81 d
Mean for cultivars					
Areda Dala Argona Maja		0.77 a 0.98 b 1.45 d 1.15 c	0.72 b 0.60 a 1.01 c 1.08 d	0.43 b 0.44 b 0.36 a 0.43 b	0.64 a 0.68 b 0.94 d 0.88 c
Mean for fertilization					
Control object Copper deficiency		1.29 b 0.88 a	1.04 b 0.66 a	0.45 b 0.39 a	0.93 b 0.64 a
Mean for cuts		1.09 c	0.85 b	0.42a	0.79 b

Explanations, see Table 1

Table 3

Water use efficiency (µmol CO_2 m^{-2} s^{-1} · m mol H_2O m^{-2} s^{-1})

Cultivars	Fertilization	1st cut	2nd cut	3rd cut	Mean
Areda	control object copper deficiency	6.70 ^{b} 5.28 ^{a}	10.02 ^{b} 16.03 ^{d}	14.26 ^{c} 14.84 ^{c}	10.33 ^{b} 12.05 ^{d}
Dala	control object copper deficiency	6.89 ^{b} 6.74 ^{b}	10.93 ^{c} 15.95 ^{d}	11.28 ^{a} 13.96 ^{c}	9.70 ^{ab} 12.23 ^{d}
Argona	control object copper deficiency	4.66 ^{a} 5.02 ^{a}	8.63 ^{a} 8.17 ^{a}	19.11 ^{e} 16.09 ^{d}	10.80 ^{c} 9.80 ^{ab}
Maja	control object copper deficiency	5.23 ^{a} 4.67 ^{a}	7.97 ^{a} 10.63 ^{bc}	23.89 ^{f} 12.38 ^{ab}	12.36 ^{d} 9.23 ^{a}
Mean for cultivars					
Areda Dala Argona Maja		5.99 ^{b} 6.81 ^{c} 4.83 ^{a} 4.95 ^{a}	13.02 ^{c} 13.44 ^{c} 8.40 ^{a} 9.30 ^{b}	14.55 ^{b} 12.62 ^{a} 17.60 ^{c} 18.14 ^{c}	11.19 ^{b} 10.96 ^{b} 10.28 ^{a} 10.80 ^{b}
Mean for fertilization					
Control object Copper deficiency		5.87 ^{b} 5.43 ^{a}	9.39 ^{a} 13.70 ^{b}	17.14 ^{b} 14.32 ^{a}	10.80 ^{a} 10.81 ^{a}
Mean for cuts		5.65 ^{a}	11.04 ^{b}	15.73 ^{c}	10.81 ^{b}

Explanations, see Table 1

cant effect on chlorophyll concentrations in leaves. Leaf greenness (SPAD) values decreased by approximately 7-8% in all regrowths under copper deficit conditions (Table 4). Cv. Maja showed the strongest response to copper deficiency, which was manifested by an 11% decrease in the chlorophyll content of leaves. At the same time, this cultivar had the highest chlorophyll concentration (on average 42.73 SPAD). The lowest chlorophyll content of leaves was recorded in the first regrowth, regardless of a cultivar. This is consistent with our earlier findings (Olszewska 2006, Olszewska et al. 2008).

Perennial ryegrass cv. Maja was characterized by the highest yield of all tested cultivars, both under optimum fertilization conditions and under copper deficit conditions (Table 5). In this cultivar a high yield corresponded with a high rate of photosynthesis and transpiration, and with the highest chlorophyll content of leaves. Deficit in soil Cu caused a yield decline in all the cultivars, but statistically significant differences were observed only with regard to cv. Areda. Dry matter yield dropped on average by around 8%, and the highest decline was noted in cv. Dala (approx. 11%). In all the analyzed cultivars the strongest response to copper deficiency was reported in the second regrowth, which was reflected by the highest yield decrease.

Table 4

Leaf greenness index (SPAD)

Cultivars	Fertilization	1st cut	2nd cut	3rd cut	Mean
Areda	control object copper deficiency	36.13 ^{cd} 33.48 ^{a}	40.85 ^{b} 38.90 ^{a}	42.03 ^{d} 40.45 ^{bc}	39.67 ^{b} 37.61 ^{a}
Dala	control object copper deficiency	35.43 ^{bc} 34.40 ^{ab}	41.05 ^{bc} 37.25 ^{a}	42.03 ^{d} 38.85 ^{a}	39.50 ^{b} 36.83 ^{a}
Argona	control object copper deficiency	39.95 ^{e} 36.88 ^{cd}	43.18 ^{d} 41.43 ^{bc}	41.58 ^{cd} 39.65 ^{b}	41.57 ^{c} 39.32 ^{b}
Maja	control object copper deficiency	41.98 ^{f} 37.85 ^{d}	48.10 ^{e} 42.2 ^{c}	45.90 ^{e} 40.38 ^{bc}	45.33 ^{d} 40.14 ^{b}
Mean for cultivars					
Areda Dala Argona Maja		34.80 ^{a} 34.91 ^{a} 34.41 ^{b} 39.91 ^{c}	39.88 ^{a} 39.95 ^{a} 42.30 ^{b} 45.15 ^{c}	41.23 ^{b} 39.64 ^{a} 40.61 ^{b} 43.14 ^{c}	38.64 ^{a} 38.17 ^{a} 40.44 ^{b} 42.73 ^{c}
Mean for fertilization					
Control object Copper deficiency		38.37 ^{b} 35.63 ^{a}	43.29 ^{b} 39.94 ^{a}	42.88 ^{b} 39.83 ^{a}	41.51 ^{b} 38.48 ^{a}
Mean for cuts		37.01 ^{a}	41.82 ^{b}	41.16 ^{b}	39.99 ^{b}

Explanations, see Table 1

Table 5

Dry matter yield ($g \cdot pot^{-1}$)

Cultivars	Fertilization	1st cut	2nd cut	3rd cut	Mean
Areda	control object copper deficiency	7.38 ^{a} 7.23 ^{a}	7.63 ^{c} 6.50 ^{ab}	5.45 ^{ab} 5.03 ^{a}	20.45 ^{bc} 18.75 ^{a}
Dala	control object copper deficiency	8.15 ^{a} 6.98 ^{a}	7.43 ^{bc} 6.40 ^{ab}	5.67 ^{ab} 5.62 ^{ab}	21.25 ^{bc} 19.00 ^{ab}
Argona	control object copper deficiency	7.50 ^{a} 7.68 ^{a}	7.13 ^{bc} 6.35 ^{a}	6.45 ^{cd} 5.98 ^{cd}	21.07 ^{bc} 20.00 ^{ab}
Maja	control object copper deficiency	8.73 ^{a} 7.93 ^{a}	7.05 ^{bc} 6.65 ^{bc}	6.70 ^{d} 6.35 ^{cd}	22.48 ^{c} 20.93 ^{bc}
Mean for cultivars					
Areda Dala Argona Maja		7.30 ^{a} 7.56 ^{a} 7.59 ^{a} 8.33 a	7.06 ^{a} 6.91 ^{a} 6.74 ^{a} 6.85 ^{a}	5.23 ^{a} 5.65 ^{a} 6.21 ^{b} 6.53 ^{b}	19.60 ^{a} 20.13 ^{a} 20.54 ^{ab} 21.70 ^{b}
Mean for fertilization					
Control object Copper deficiency		7.93 ^{a} 7.45 ^{a}	7.31 ^{b} 6.48 ^{a}	6.07 ^{b} 5.74 ^{a}	21.31 ^{b} 19.67 ^{a}
Mean for cuts		7.69 ^{c}	6.89 ^{b}	5.91 ^{a}	20.49 ^{d}

Explanations, see Table 1

CONCLUSIONS

1. Copper deficiency significantly decreased the rate of photosynthesis and transpiration, chlorophyll concentration in leaves and the yield of all the investigated cultivars.

2. Perennial ryegrass cv. Maja was found to be most resistant to copper deficiency – it was characterized by a high rate of photosynthesis and transpiration, and by the highest chlorophyll content. The yield of cv. Maja attained under copper deficit conditions was comparable to that of other cultivars grown under control conditions.

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