Foliar fertilization with molybdenum in sunflower (*Helianthus annuus* L.)

P. Škarpa[1], E. Kunzová[2], H. Zukalová[3]

[1]*Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Mendel University in Brno, Brno, Czech Republic*
[2]*Crop Research Institute, Prague, Czech Republic*
[3]*Department of Crop Production, Czech University of Life Sciences Prague, Prague, Czech Republic*

ABSTRACT

The objective of the vegetation experiment established in 2008–2011 was to explore the effect of the time and dose of foliar molybdenum (Mo) application on the yield and quality of sunflower. Four treatments were established in the experiment: (1) control – not fertilised with Mo; (2) application of 125 g Mo/ha in the growing stage of 4 developed leaves (V-4); (3) application of 125 g Mo/ha at the beginning of elongation growth (R-1), and (4) split rate of Mo application of 62 g Mo/ha at stage V-4 (4 developed leaves) and 62 g Mo/ha at stage R-1 (terminal bud forms). Foliar application of molybdenum increased the biomass production of sunflower plants and its content in dry matter. A statistically significant effect of molybdenum foliar application on sunflower yields was found. Foliar application of Mo up to a dose of 125 g Mo/ha at the beginning of vegetation (stage V-4) and developmental stage R-1 increased yields of achenes. The relative increase in the oil content after foliar nutrition was not significant and ranged between 1.4% and 2.6%. Oil production increased due to increased yields and stabilised oil content. Foliar application of molybdenum had no effect on the content of oleic acid.

Keywords: sunflower nutrition; foliar nutrition; achene yield; oil content; fatty acid

Molybdenum (Mo) belongs among essential microelements. Total Mo content of soil varies from 0.01 to 17.0 mg/kg (Kabata-Pendias 2011). Most agricultural soils contain a relatively low amount of molybdenum by comparison, with an average of 2.0 mg/kg total molybdenum and 0.2 mg/kg available molybdenum (Mengel and Kirkby 2001). The availability of molybdenum for plant growth is strongly dependent on the soil pH, concentration of adsorbing oxides (e.g. Fe oxides), extent of water drainage, and organic compounds found in the soil colloids.

Molybdenum is essential for most organisms and occurs in more than 60 enzymes catalyzing diverse oxidation-reduction reactions in plant metabolism (Zimmer and Mendel 1999). Mo is a constituent of nitrogenase (NA) and nitrate reductase (NR), required for the assimilation of soil nitrates. Therefore, the function of Mo is closely related to plant nitrogen metabolism, and Mo deficiency is manifested as deficiency of plant N (Pollock et al. 2002). The critical deficiency concentration in most crop plants is quite low, normally between 0.1 and 1.0 mg Mo/kg dry tissue (Gupta and Lipsett 1981). Because molybdenum is very mobile within the plant, its deficiency can be observed in the whole plant, most often in the middle of the plant or on old leaves by getting yellow

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QH81271.

Table 1. Agrochemical characteristics of the soil

Year	$pH/CaCl_2$	Content of nutrients (mg/kg dry matter soil)				
		P	K	Ca	Mg	available Mo
2008	6.2	91	254	2 672	244	0.086
2009	6.2	66	179	4 477	313	0.069
2010	6.7	63	111	2 321	164	0.096
2011	6.8	78	206	2 864	262	0.104

or yellow-green colour (Hamlin 2007). In the case of dicotyledonous species, a drastic reduction in leaf size and irregularities in leaf blade formation are the most typical visible symptoms caused by local necrosis in the tissue and insufficient differentiation of vascular bundles at an early stage of leaf development (Gupta and Lipsett 1981). Molybdenum fertilization through foliar sprays can effectively supplement internal Mo deficiencies and rescue the activity of molybdo-enzymes (Kaiser et al. 2005).

Since not much is known about the nutritional effects of Mo foliar application in sunflower nutrition, field experiments were conducted at the Mendel University in Brno to investigate the response of sunflower to different times and doses of Mo foliar application on achenes yield and quality.

MATERIAL AND METHODS

Precise small-plot experiment with sunflower (*Helianthus annuus*) was established at the School Farm of Mendel University of Agriculture and Forestry in Brno in Žabčice (48°57'26''N, 16°36'18''E) in 2008 and 2009 and Agricultural Cooperative Ivaň in the cadastre of Vranovice (48°57'26''N, 16°36'18''E) in 2010 and 2011. In the experiment we explored the effect of the molybdenum rate and time of application on sunflower production and achene quality.

The content of nutrients in the soil analysed prior to the establishment of the experiment (Table 1) was at a satisfactory to very high level. P, K, Ca and Mg were estimated by the method of Jones (1990), with the soil extracted with a solution Mehlich III. Available Mo in soil had been determined by Grigg (1953). The soil reaction ($pH/CaCl_2$) was slightly acid (2008 and 2009) to neutral (2010 and 2011).

In all the years of the experiment we used the hybrid Orasole (high oleic sunflower). Prior to sowing the plot was fertilized to a rate of 100 kg N/ha (this rate included the content of N_{min} determined before sowing). On sowing the inter-row distance was 75 cm, the seeds in the row were spaced 20 cm apart to a depth of 4–6 cm. After sowing the plot was compacted and pre-emergence application of herbicides followed.

After emergence of the plants a small-plot field experiment was established (4.5 m × 15 m plot area). Mo application was done in the form of foliar nutrition in developmental stage V-4 – 4 developed leaves and stage R-1 – terminal bud forms a miniature floral head rather than a cluster of leaves in combinations and rates given in Table 2. Mo was applied in the form of sodium molybdate (Na_2MoO_4). Each treatment was repeated 4 times.

The content of dry matter and the levels of nutrients (N, P, K, Ca, Mg and Mo) were determined in plant mass in developmental stages V-4, R-1 (approx. 12 days after foliar application in V-4 developmental stage) and R-2 (immature bud elongates 0.5 cm to 2.0 cm above the nearest leaf attached to the stem – approx. 12 days after foliar application in R-1 developmental stage). The samples of plant mass were dried at a temperature of 60°C, then crushed in a grinder, and homogenized. The resultant crushed plant mass was mineralized using a mixture of H_2SO_4 and H_2O_2 (Zbíral 2005).

Table 2. Treatments of the experiment

Treatment	Dose of Mo (g/ha)	Time of application*
Control	0	no fertilizers
Mo1	125	V-4
Mo2	125	R-1
Mo3	62	V-4
	62	R-1

*stages of sunflower development (Schneiter and Miller 1981)

Table 3. Dry weight and plant nutrients concentration in the V-4 stage of sunflower development

Dry weight of plant (g per plant)	N	P	K	Ca	Mg	Mo (mg/kg DM)
	(% DM)					
3.01 ± 0.33	4.27 ± 0.33	0.40 ± 0.04	5.04 ± 0.30	1.94 ± 0.43	0.77 ± 0.11	0.38 ± 0.03

Values show mean of experiments 2008–2011 ± standard error of the mean. DM – dry matter

The amount of N in the mineralized sample was determined using the Kjeldahl method. The content of P in the extract was determined colorimetrically using a Unicam 8625 UV/Vis spectrometer (ATI Unicam, Cambridge, UK). The levels of K, Ca, Mg and Mo were determined in (the mineralized sample using the Atomic Absorption Spectrometry (AAS) with the ContrAA 700 instrument (Analytik Jena AG, Jena, Germany).

Sunflower was harvested when it reached physiological ripeness. Yield of achenes, oil content, oil production and levels of fatty acids (palmitic, stearic, oleic and linoleic) in the achenes were evaluated after harvest. Oil content was determined using the Soxhlet method based on the NMR extraction of sunflower achenes in a continuous flow extractor Minispec mq series TD-NMR (Bruker Corporation, Ettlingen, Germany). The levels of fatty acids (FA) were determined as methyl esters using Agilent 6890 Series Gas Chromatograph (Agilent Technologies, Inc., Wilmington, USA).

The Statistica 9 programme (Tulsa, USA) was used for the determination of the overall statistical characteristics. Arithmetic means were calculated when evaluating the results of experiments from 2008–2011. To elaborate the significance of differences among the arithmetic means of each characteristic we used the mono-factor and two-factor ANOVA followed by testing at a 95% ($P < 0.05$), 99% ($P < 0.01$) and 99.9% ($P < 0.001$) level of significance using the Fischer's *LSD* test.

RESULTS AND DISCUSSION

According to Cerkal et al. (2011), results of inorganic analyses in stage V-4 (Table 3) showed that sunflower had a sufficient supply of all macro biogenic nutrients.

However the soil had a low content of Mo and was therefore not able to provide the plant with an amount sufficient according to the plant analyses (the optimal Mo content ranges between 0.4 and 1.0 mg/kg DM).

Molybdenum foliar application in stage V-4 increased Mo content in the plant biomass at R-1 stage of sunflower development (Table 4). The rate of 62 g Mo/ha (Mo3) significantly increased ($P < 0.05$) the concentration of Mo in leaves (Table 4) comparably to the treatment with supplementary 125 g Mo/ha (Mo1). Content of molybdenum in tissue (leaves) increased more than tenfold. Foliar application of molybdenum increased its concentration in tissue of winter wheat (Brennan and Bolland 2007) and

Table 4. Dry weight and plant nutrients concentration in the R-1 stage of sunflower development

Part of plant	Treatment	Dry weight of plant (g/plant)	N	P	K	Ca	Mg	Mo (mg/kg DM)
			(% DM)					
Leaves	control	8.68 ± 1.51	4.13 ± 0.42	0.40 ± 0.04	4.23 ± 0.39	2.05 ± 0.63	0.89 ± 0.20	0.96^a ± 0.06
	Mo1	10.54 ± 2.13	4.32 ± 0.25	0.44 ± 0.03	4.16 ± 0.09	1.81 ± 0.56	0.84 ± 0.17	9.29^b ± 0.09
	Mo3	10.03 ± 2.29	4.37 ± 0.21	0.45 ± 0.02	4.37 ± 0.17	2.17 ± 0.75	0.79 ± 0.21	6.13^b ± 0.13
Stems	control	3.77 ± 1.41	2.09 ± 0.38	0.28 ± 0.01	5.87 ± 1.04	1.18 ± 0.22	0.81 ± 0.17	0.45^a ± 0.03
	Mo1	4.97 ± 1.41	2.18 ± 0.16	0.33 ± 0.06	5.80 ± 0.89	1.10 ± 0.31	0.83 ± 0.25	0.97^a ± 0.03
	Mo3	5.00 ± 1.43	2.13 ± 0.15	0.31 ± 0.04	6.17 ± 0.79	1.17 ± 0.38	0.77 ± 0.16	0.78^a ± 0.02

Means followed by the different letters are significantly different (mg Mo/kg DM – $P < 0.05$). Values show mean of experiments 2008–2011 ± standard error of the mean. DM – dry matter

Table 5. Dry weight and plant nutrients concentration in the R-2 stage of sunflower development

Part of plant	Treatment	Dry weight of plant (g/plant)	N	P	K	Ca	Mg	Mo (mg/kg DM)
			(% DM)					
Leaves	control	27.56 ± 4.20	3.02 ± 0.28	0.36 ± 0.02	3.35 ± 0.65	1.74 ± 0.53	0.83 ± 0.16	0.82^{a} ± 0.05
	Mo1	38.29 ± 3.17	3.05 ± 0.22	0.38 ± 0.01	3.55 ± 0.42	1.75 ± 0.52	0.76 ± 0.22	3.85^{b} ± 0.15
	Mo2	34.26 ± 4.88	3.20 ± 0.13	0.39 ± 0.05	3.73 ± 0.16	1.66 ± 0.44	0.68 ± 0.18	10.16^{c} ± 0.14
	Mo3	32.00 ± 0.98	3.51 ± 0.13	0.39 ± 0.01	3.34 ± 0.36	1.69 ± 0.47	0.82 ± 0.20	12.76^{c} ± 0.08
Stems	control	26.15 ± 2.46	1.10 ± 0.38	0.25 ± 0.052	4.49 ± 0.57	0.83 ± 0.20	0.71 ± 0.19	0.31^{a} ± 0.02
	Mo1	35.09 ± 3.44	1.24 ± 0.31	0.26 ± 0.01	4.47 ± 0.78	0.77 ± 0.18	0.69 ± 0.20	0.43^{a} ± 0.04
	Mo2	29.66 ± 4.58	1.48 ± 0.14	0.30 ± 0.03	4.60 ± 0.47	0.76 ± 0.22	0.61 ± 0.23	0.65^{ab} ± 0.03
	Mo3	30.74 ± 3.47	1.40 ± 0.17	0.25 ± 0.01	4.32 ± 0.64	0.79 ± 0.19	0.71 ± 0.20	0.93^{b} ± 0.04

Means followed by different letters are significantly different (mg Mo/kg DM – $P < 0.05$). Values show mean of experiments 2008–2011 ± standard error of the mean. DM – dry matter

common bean (Silva et al. 2012). Foliar nutrition did not significantly affect molybdenum content in the stem, although its contents were increased almost two times.

Molybdenum application also increased the uptake of macro biogenic nutrients (especially N), as can be seen in analyses of plants taken in stage R-1 (Table 4). According to Ayala et al. (2005), treatment with a foliar application of Ca + Mo increased the N content of poinsettia by 26% compared with the control. Also Silva et al. (2012) reported increased nitrogen content in common bean leaves and Bagheri and Jafari (2012) in plant of barley due to foliar application of molybdenum.

Foliar nutrition at the beginning of vegetation also increased the dry matter weight of leaves (by 15.6–21.4%) and stems (by 31.8–32.6%). A shortage of molybdenum in winter wheat (Yu et al. 1999) nutrition inhibits plant growth and reduces dry matter production. According to Liu et al. (2005), molybdenum promoted yield of soybean above-ground biomass.

The positive effect of molybdenum fertilization in stages V-4 and R-1 on dry matter production (Mo1–Mo3) is obvious from the results of analyses of plants in stage R-2 (Table 5). The average dry matter weights of leaves and stems were increased by 26.5% and 21.7%, respectively. Foliar application

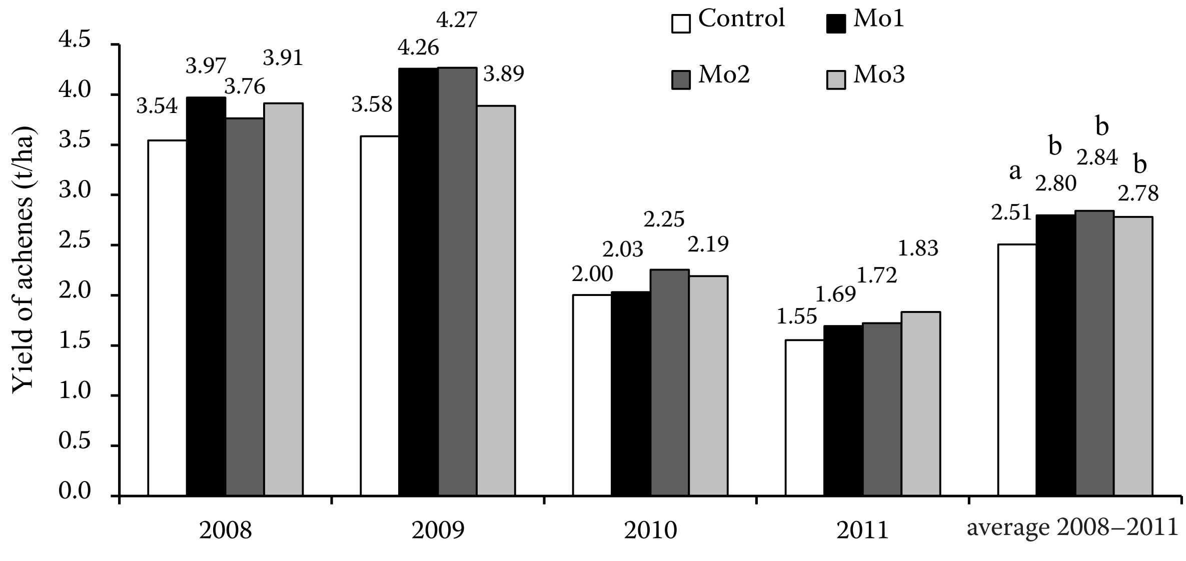

Figure 1. Effect of different leaf fertilization on achenes yield. Means followed by the different letters are significantly different (Yield of achenes – $P < 0.05$). Values show mean of experiments 2008–2011

Table 6. Effect of different leaf fertilization on oil content and oil production

Treatments	Oil content (%)	Oil production (t/ha)
Control	$44.83^{a} \pm 0.61$	$1.191^{a} \pm 0.083$
Mo1	$45.98^{a} \pm 0.70$	$1.387^{b} \pm 0.021$
Mo2	$45.47^{a} \pm 0.76$	$1.384^{b} \pm 0.104$
Mo3	$45.48^{a} \pm 0.85$	$1.362^{b} \pm 0.082$

Means followed by the different letters are significantly different ($P < 0.05$). Values show mean of experiments 2008–2011 ± standard error of the mean

in stage R-1 (Mo2 and Mo3) increased molybdenum content more than 10 fold, i.e. statistically significantly ($P < 0.05$) in sunflower leaves when compared with treatment without Mo application.

Molybdenum applications had a positive effect on achene production in all fertilisation treatments (Figure 1). Yield increase due to foliar application of molybdenum in oilseeds (winter rape) was found according to Grzebisz et al. (2010). Stanislawska-Glubiak (2008) reported a significant increase of rape yield after fertilization of 60 and 120 g Mo about 2.7% to 3.4% in slightly acid and neutral soil. The foliar application of Mo up to a dose of 35 g/ha increased the yield of wheat (Zoz et al. 2012). Achene yields increased statistically significantly ($P < 0.05$) after foliar applications of molybdenum in the range from 10.8% (Mo3) to 13.3% (Mo2). According to Valenciano et al. (2011), foliar Mo application of chickpea resulted in a higher number of pods per plant and a higher yield (increased from 1.87 to 2.45 g/plant). Also Silva et al. (2012) reported increased grains yield of common bean with foliar application of Mo in the dose of 60 g/ha. Molybdenum application significantly increased grain yields for most cultivars of winter wheat (Yu et al. 1999).

Although in all the treatments foliar molybdenum nutrition increased the oil content (ranging between 1.4% and 2.6%), this increase was not statistically significant ($P < 0.05$). In all the treatments the oil content ranged between 44.83% and 45.98% (Table 6). On the contrary, Samul and Bhattacharyya (1980) reported lower oil content due to molybdenum application.

Due to higher yields and stabilised oil content the resulting effect of fertiliser application was a higher oil production (t/ha); the fertilisation combinations explored statistically significantly ($P < 0.05$) exceeded the control treatment, most of all with molybdenum application in stages V-4 (Mo1) and R-1 (Mo2) on average by 16.3%.

In terms of quantitative parameters of sunflower we evaluated the representation of fatty acids in the oil; their composition is given in Table 7. Among the most important fatty acids we rank oleic acid (in sunflower of the 'high oleic' type the proportion of oleic acid should be at least 82%). The results of analysis showed that the content of oleic acid ranged between 84.89% and 87.29% and foliar nutrition did not have a significant ($P < 0.05$) effect on its amount. Application of Mo increased significantly ($P < 0.05$) only the content of stearic acid which ranged between 5.6% and 11.3%, and was the highest in the treatment with molybdenum application at the beginning of vegetation (Mo1). On the contrary, stearic acid content in seeds of rape was decreased by application of Mo fertilizer (Liu et al. 2009).

REFERENCES

Ayala J., Castillo A.M., Colinas M.T., Pineda J. (2005): Effect of foliar application of calcium, boron, and molybdenum in nutrient content of poinsettia plants. HortScience, *40*: 1086.

Bagheri A.R., Jafari A.R. (2012): Effect of salinity and molybdenum application on photosynthesis, nitrogenase activity and

Table 7. The content of fatty acid in sunflower oil

Treatments of fertilization	C 16:0	C 18:0	C 18:1	C 18:2
Control	$3.39^{a} \pm 0.10$	$3.20^{a} \pm 0.08$	$87.01^{a} \pm 1.24$	$6.28^{a} \pm 1.26$
Mo1	$3.46^{a} \pm 0.11$	$3.56^{b} \pm 0.08$	$84.89^{a} \pm 1.65$	$7.98^{a} \pm 1.59$
Mo2	$3.49^{a} \pm 0.14$	$3.40^{ab} \pm 0.09$	$85.12^{a} \pm 1.27$	$7.84^{a} \pm 1.15$
Mo3	$3.30^{a} \pm 0.12$	$3.38^{ab} \pm 0.06$	$87.29^{a} \pm 1.12$	$5.93^{a} \pm 1.02$

C 16:0 – palmitic; C 18:0 – stearic; C 18:1 – oleic; C 18:2 – linoleic. Means followed by the different letters are significantly different ($P < 0.05$). Values show mean of experiments 2008–2011 ± standard error of the mean

yield of barley inoculated with *Azosprillium brasilense*. Cereal Research Communications, *40*: 235–245.

Brennan R.F., Bolland M.D.A. (2007): Increased concentration of molybdenum in sown wheat seed decreases grain yield responses to applied molybdenum fertilizer in naturally acidic sandplain soils. Journal of Plant Nutrition, *30*: 2005–2019.

Cerkal R., Kamler J., Škarpa P., Pokorný R., Mareček V., Fajman M., Muška F. (2011): Methods of cultivation and important factors affecting the yield and quality of sunflower. In: Hughes V.C. (ed.): Sunflowers: Cultivation, Nutrition, and Biodiesel Uses. Nova Science Publishers, Inc., New York, 47–98.

Grigg J.L. (1953): Determination of the available molybdenum in soils. New Zealand Journal of Science and Technology, *34*: 405–410.

Grzebisz W., Lukowiak R., Biber M., Przygocka-Cyna K. (2010): Effect of multi-micronutrient fertilizers applied to foliage on nutritional status of winter oilseed rape and development of yield forming elements. Journal of Elementology, *15*: 477–491.

Gupta U.C., Lipsett J. (1981): Molybdenum in soils, plants and animals. Advances in Agronomy, *34*: 73–115.

Hamlin R.L.: (2007): Molybdenum. In: Barker A.V., Philbeam D.J. (eds.): Handbook of Plant Nutrition. Taylor and Francis Group, New York, 375–394.

Jones J.J.B. (1990): Universal soil extractants: Their composition and use. Communications in Soil Science and Plant Analysis, *21*: 1091–1101.

Kabata-Pendias A. (2011): Trace Elements in Soils and Plants. 4th Edition. CRC Press, Taylor and Francis Group, Boca Raton, 505.

Kaiser B.N., Gridley K.L., Brady J.N., Phillips T., Tyerman S.D. (2005): The role of molybdenum in agricultural plant production. Annals of Botany, *96*: 745–754.

Liu P., Yang Y.S., Xu G.D., Fang Y.H., Yang Y.A., Kalin R.M. (2005): The effect of molybdenum and boron in soil on the growth and photosynthesis of three soybean varieties. Plant, Soil and Environment, *51*: 197–205.

Liu H., Hu C., Sun X., Tan Q., Nie Z., Su J., Liu J., Huang H. (2009): Interactive effects of molybdenum and phosphorus fertilizers on grain yield and quality of *Brassica napus*. Journal of Food, Agriculture and Environment, *7*: 266–269.

Mengel K., Kirkby E.A. (2001): Principles of Plant Nutrition. 5th Edition. Kluwer Academic Publishers, Dordrecht, 849.

Pollock V.V., Conover R.C., Johnson M.K., Barber M.J. (2002): Bacterial expression of the molybdenum domain of assimilatory nitrate reductase: Production of both the functional and nonfunctional molybdenum-containing domain and the nonfunctional tungsten analog. Archives of Biochemistry and Biophysics, *403*: 237–248.

Samul R.C., Bhattacharyya P. (1980): Effect of soil and foliar application of nitrogen, potassium and molybdenum on oil content and yield and chemical composition of sunflower. Journal of the Indian Society of Soil Science, *28*: 193–198.

Schneiter A.A., Miller J.F. (1981): Description of sunflower growth-stages. Crop Science, *21*: 901–903.

Silva E.B., Santos S.R., Fonseca F.G., Tanure L.P.P., Freitas J.P.X. (2012): Foliar application of molybdenum in irrigated common bean cultivated in the Northern Minas Gerais, Brazil. Bioscience Journal, *28*: 64–71.

Stanislawska-Glubiak E. (2008): The influence of soil reaction on the effects of molybdenum foliar fertilization of oilseed rape. Journal of Elementology, *13*: 647–654.

Valenciano J.B., Marcelo V., Miguelez-Frade M.M. (2011): Effect of different times and techniques of molybdenum application on chickpea (*Cicer arietinum*) growth and yield. Spanish Journal of Agricultural Research, *9*: 1271–1278.

Yu M., Hu C.X., Wang Y.H. (1999): Influences of seed molybdenum and molybdenum application on nitrate reductase activity, shoot dry matter, and grain yields of winter wheat cultivars. Journal of Plant Nutrition, *22*: 1433–1441.

Zbíral J. (2005): Plant Analysis. JPP Central Institute for Supervising and Testing in Agriculture, Brno, 192.

Zimmer W., Mendel R. (1999): Molybdenum metabolism in plants. Plant Biology, *1*: 160–168.

Zoz T., Steiner F., Testa J.V.P., Seidel E.P., Fey R. Castagnara D.D., Zoz A. (2012): Foliar fertilization with molybdenum in wheat. Ciências Agrárias, *33*: 633–638.

Received on October 2, 2012
Accepted on January 23, 2013

Corresponding author:

Ing. Petr Škarpa, Ph.D., Mendelova univerzita v Brně, Agronomická fakulta, Ústav agrochemie, půdoznalství, mikrobiologie a výživy rostlin, Zemědělská 1, 613 00 Brno, Česká republika
phone: + 420 545 133 345, fax: + 420 545 133 096, e-mail: petr.skarpa@mendelu.cz