The effect of application of copper fungicides on photosynthesis parameters and level of elementary copper in hops

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ABSTRACT

Photosynthesis and transpiration rates in the interval of 30 min before and 30 min after copper fungicide application show an increase from the level of 5.0 to 7.0 μmol $CO_2/m^2/s$ and 0.75 to 1.00 mmol $H_20/m^2/s$. Long-term measurements show that the increase of photosynthesis rate after copper application is temporal and fades away after 10–14 days. No stress response was induced after the application of copper fungicides. Contents of copper in hop cones are up to 500 mg/kg if total amount of applied copper does not exceed 15 kg/ha. Contents of copper on leaves are 2–5 times higher at the same application dose. Application of 5 kg copper per one hectare of vigorous growth of Agnus variety increases content of copper on hop leaves by 1000 mg/kg at least. The same amount of copper increases its content in hop cones by 300 mg/kg at the ripening period. Tight correlation between the amount of copper applied and its content in hops does not exist. Elementary copper from leaves is brought into harvested hops in the form of biological admixtures. Copper content in hop cones shows a decreasing trend, which is given by gradual increase of cones size at the ripening period. Similar trend on hop leaves shows that washing off and dissolving of copper compounds by atmospheric water can participate in this process as well. Common content of copper in untreated hop cones and leaves is up to 20–25 mg/kg.

Keywords: downy mildew; copper; photosynthesis rate; transpiration rate

Downy mildew (*Pseudoperonospora humuli* L.) is the most important fungal disease of hop. It can cause high losses in yield and quality of harvested hops. First symptoms of infestation appear on young sprouts in early spring. Leaves are of green-yellow colour, stunt and due to shortening of internodia become accumulated. They are commonly call spikes. Spikes are the main source for further spreading of the disease in the course of hop maturation (Vostřel 2010). An important regulation factor of downy mildew occurence in the phase of flowering and cone forming is application of copper fungicides. Disease control consists in disrupting the function of the cellular proteins of fungi and bacteria (Marsh 1937). In spite of they belong to the oldest known pesticides copper fungicides still have great importance in plants protection. Copper is an essential microelement needed by plants (Sommer 1931). Appr. 70% of copper is localised in chloroplasts where it acts as chlorophyll stabilizer. Copper acts as acceptor or electron donor and becomes a component of many oxidation-reduction systems. It parcipates on lignin biosynthesis that stabilises cell walls. Copper as protein component plays important role in photosynthesis processes and cell respiration. It is indispensable for symbiotic relationship between nitrogen fixing bacteria and roots of bean plants as well (Yruela 2005). Plant requirement for this chemical element is small and moves in the range of 2 to 25 ppm. Portner (2006) presents total copper take-off 4 054 mg/ha of hop garden, from which falls 581 g/ha to cones, the rest for bine and leaves. Copper is one of the most widely reported heavy metal soil polutants in the world (Jensen-Spaulding 2004, Takáč 2009). The toxicity of copper depends on soil properties and the amount of available forms in the soil (Kunito

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Table 1. Characteristics of used copper fungicides

Pesticide	Composition	Copper content	Application amount	Amount Cu/ha (kg/treatment)
Kuprikol 50	copper oxychloride $CuCl_2 \cdot 3\ Cu(OH)_2$	500 g/kg	0.50%/2000 L	5
Kuprikol 250 SC	copper oxychloride $CuCl_2 \cdot 3\ Cu(OH)_2$	250 g/L	1.0%/2000 L	5
Curzate K	copper oxychloride + cymoxanil $CuCl_2 \cdot 3\ Cu(OH)_2$	460 g/L	0.30%/2000 L	2.8
Cuproxat SC	basic copper sulphate $Cu\ SO_4 \cdot 3\ Cu(OH)_2 \cdot 1/2\ H_2O$	190 g/L	0.75%/2000 L	2.9

1999, Rajapaksha 2004). Komarek (2009) showed that application of copper-based fungicides to hop fields resulted into soil Cu concentrations well above 150 mg/kg. Soil pH proved to be the dominant factor having an influence on vertical mobility of copper in studied soils.

There are few references describing the measurement of photosynthesis in hop. Peat (1974) showed that mature cones had measurable photosynthetic activity, which rarely exceed respiratory loss. Kenny (2005) studied mean photosynthesis rates in 40 hop genotypes originating from North America region. He found out the mean photosynthesis rate 16.2 μmol $CO_2/m^2/s$ and range of photosynthesis performance in the interval of 9–23 μmol $CO_2/m^2/s$. Hnilickova (2007) determined saturation irradiance and photosynthetic capacity for several Czech hop varieties by gasometric method. Photosynthesis is affected by many environmental stresses that limit the potential growth of canopies. Many stresses are caused directly or indirectly by water shortages, mineral requirements, particularly nitrogen. Fungal diseases and pests may also induce stresses by reducing the green leaf area, the sap flow, or by altering plant metabolism (Baret 2007). Theoretically, application of pesticides by spraying on leaf surface can bring about stress conditions as well.

In brewing technology copper belongs to the most important metal elements. It plays the relevant role in efficiency of many malt enzymes. It is esential for yeast growth in low concentrations but it is toxic in high ones. Copper has important role in formation of colloidal hazes (Cejka 1989). Sensorial ageing of beer significantly increases in the presence of Cu^{2+} ions at concentrations below 100 μg/L. Copper ions catalyze oxidation reactions in the presence of prooxidants like cystein and 1,2,3-trihydroxy-polyphenols (Irwin 1991). Copper and iron ions participate in non enzymatic oxidation of unsaturated fatty acids (Bamforth 1999). Content of copper determined by AAS method in Pilsener type beers was found in the interval of 19 to 35 μg/L (Mäder 1997). Authors state that brewing technology is the decontamination process from the point of view of copper and other heavy metals content. Dostálek (2001), who assessed content of heavy metals in 200 beer samples by chronopotentiometric method came to the similar conclusions. Average copper content in the file of beers was 32.6 μg/L with maximal value 93 μg/L. Beer is considered to be very pure beverage from the heavy metal contents point of view.

Hop is an important commodity used in food industry. Therefore, hygienic limits of pollutants (heavy metals) have to be followed and respected (Public Notice of Ministry of Health Nr. 298/1997). It is necessary to deal with this topic particularly for metals, which are contained in pesticides used for chemical control of hop. Maximum residue level (MRL) of elementary copper in hops and hop products valid in EU is 1 000 mg/kg. In USA and Japan they are not even fixed. Within the scope of research project 'Integrated hop production' repeated tests of multiple copper fungicides application were performed with the aim of determination its impact on photosynthesis rate and on the content of elementary copper in hops in the course of 2008–2010 vegetation seasons.

Table 2. Application terms of fungicides and dosage of elementary copper

Application	2008		2009		2010	
	date	Cu (kg/ha)	date	Cu (kg/ha)	date	Cu (kg/ha)
I.	22.7.	5.0	27.7.	5.0	13.7.	2.8
II.	12.8.	5.0	14.8.	2.9	1.8.	5.0
III.	27.8.	5.0	–	–	22.8.	5. 0
Cu total		15.0		7.9		12.8

MATERIAL AND METHODS

Kuprikol 50, Kuprikol 250 SC, Cuproxat SC and Curzate K were used for the tests in the compliance with methodical hints. Detailed characteristics of used pesticides are summarized in Table 1. Utilisation of several copper fungicides which is commonplace in practice, was given by current availability on the market and price. Copper fungicides were applied on Czech hop variety Agnus with the help of common mistblowers (nozzle type Albuz, spraying pressure 1.0 MPa, driving speed 4 km/hod, water consumption 2000 L/ha). The experimental hop garden was divided into 3 parts, each of 0.3 ha acreage. The first treatment was applied on the whole plot, the second one on 2/3 of the experimental area and the third one on 1/3 of the area. Initial intention of three applications was realised only in 2008. During subsequent years only two applications were carried out due to early harvest (2009) or muddy terrain (2010). Kuprikol 50 was used in 2008, fungicides Kuprikol 250 SC and Cuproxat SC were used in 2009. In 2010 Kuprikol 250 SC was applied. With respect to high infection pressure of downy mildew Curzate K was applied out of plan on July 13^{th} 2010. The exceptional application of copper increased its contents on leaves (109 mg/kg) at the start of the test. Application terms and amounts of elementary copper after each treatment expressed in kg/ha are summarized in Table 2. In 2008 totally 15 kg of elementary copper was applied in three treatments, in 2009 7.9 kg and 12.8 kg of copper in 2010.

In the period 2008–2010 photosynthesis and transpiration rates were measured in intact leaves with the commercial handheld infrared analyser LCpro+ (ADC Bio Scientific Ltd., UK) with a leaf chamber that enables measurements at an irradiance PHAR (400–700 nm) in the range of 0–2000 μmol/m^2/s and at the temperature of –5°C to +50°C. Measurements were made in regular intervals allowing the conditions to become fixed in the measuring chamber (23°C, 600 nm of irradiation) at the day time from 6:00 till 10:30 h. Measurement of one leaf tooked 25 min giving 25 raw data about photosynthesis and transpiration rates. Average values of measured parameters were expressed as arithmetic mean after exclusion of outliers. Selected lateral leaves at the hight of 3 m of bine were free of physiological, pest and disease damage. Leaves were of comparable phenological and physiological age. Plants selected for photosynthetical measurements were located 15 m from the margin of hop garden at least due to simulation of comparable ilumination and shading. Ten plants of similar developmental stage were selected and marked in each experimetal plot. Similarly laterals, which were repetitively measured in the course of whole vegetation season, were marked as well. Photosynthetical parametres were measured in one leaf per plant. Results of 5 plants with the best conformity of photosynthesis and transpiration rates were included into the overall evaluation.

Table 3. Contents of elementary copper in hop cones (mg/kg) after fungicide applications (I.–III.)

Date	2008						2009					2010				
	I.		II.		III.		date	I.		II.		date	I.		II.	
	days	content	days	content	days	content		days	content	days	content		days	content	days	content
21.7.	control	–	–	–	–	–	25.7.	control	6	–	–	29.7.	control	109	–	–
22.7.	2 h	–	–	–	–	–	27.7.	2 h	371	–	–	2.8.	1	312	–	–
24.7.	2	–	–	–	–	–	28.7.	1	302	–	–	4.8.	3	316	–	–
28.7.	6	–	–	–	–	–	31.7.	4	286	–	–	8.8.	7	219	–	–
31.7.	9	–	–	–	–	–	3.8.	7	395	–	–	16.8.	14	176	–	–
8.8.	17	405	–	–	–	–	6.8.	10	417	–	–	23.8.	22	208	1	482
13.8.	23	–	1	–	–	–	11.8.	15	335	–	–	30.8.	29	175	8	505
14.8.	24	201	2	504	–	–	14.8.	–	–	2 h	489	6.9.	36	153	15	491
18.8.	28	133	6	487	–	–	17.8.	21	415	3	501	10.9.	40	144	19	323
25.8.	35	122	13	309	–	–	24.8.	28	429	10	556	–	–	–	–	–
28.8.	38	93	16	247	1	834	28.8.	32	350	14	456	–	–	–	–	–
1.9.	42	86	20	143	5	705	–	–	–	–	–	–	–	–	–	–
5.9.	46	108	24	201	9	471	–	–	–	–	–	–	–	–	–	–

Spraying tests were started in the second half of July and lasted till the first decade of September. Samples of appr. 30 leaves and 25–30 g of hop cones were taken after each treatment randomly from 10 plants at height of 2–6 m in approx. one week intervals. Time dependence of copper contents on leaves and in cones were obtained for each application. Drying of plant material was peformed at ambient temperature in a dark room. Dry samples of cones and leaves were analysed in an authorised laboratory for the content of elementary copper by ICP-AES method. Samples were mineralised by microwave decomposition in the environment of nitric acid and hydrogen peroxide. Detection limit was 1.5 mg Cu/kg. Results of copper content in hops (Tables 3–4) are expressed as arithmetic mean of duplicate analyses.

RESULTS AND DISCUSSION

Photosynthesis and transpiration rates in the interval of 30 min before and 30 min after copper fungicide application are shown on Figures 1–2. Determined data show that photosynthesis and transpiration rates increased after copper treatment from the level of 5.0 to 7.0 μmol $CO_2/m^2/s$ and 0.75 to 1.00 mmol $H_20/m^2/s$ respectively. It can be explained by suplement of copper ion to the photosynthetic system of leaf on the supposition that leaves of hop plant are able to accept copper cation after foliar application.

Results of repeated measurements of photosynthesis and transpitation rates of hop plants in the course of 2010 vegetation season are shown on Figures 3–4. The highest photosynthesis rates in the range of 7–9 μmol $CO_2/m^2/s$ were determined in August, i.e. the period of cone forming and hop maturation (BBCH 75–89). Distinct reduction of photosynthesis rates was found out at the beginning of September shortly before the crop harvest. Transpiration rates (0.8–1.2 mmol $H_20/m^2/s$) show similar trend of maximal values during August as well. Comparable photosynthesis and transpiration rates were measured also in other vegetation seasons. Long-term measurements show that increase of photosynthesis rate after copper application is temporal and fades away after 10–14 days. Changes in photosynthesis rates reflect current weather conditions and development stage of hop plants. Photosynthetic measurements showed that no stress response was induced after application of copper fungicides.

Copper contents in hop cones and on leaves are summarized in Tables 3–4. Contents of elementary copper in cones after the first application dose moved in the interval of 300 to 400 mg/kg. The second copper treatment increased its content to the level of 500 mg/kg. The highest content of copper in cones 834 mg/kg was found in 2008 im-

Table 4. Contents of elementary copper on hop leaves (mg/kg) after fungicide applications (I.–III.)

2008							2009					2010				
Date	I.		II.		III.		date	I.		II.		date	I.		II.	
	days	content	days	content	days	content		days	content	days	content		days	content	days	content
21.7.	control	13	–	–	–	–	25.7.	control	15	–	–	29.7.	control	383	–	–
22.7.	2 h	1778	–	–	–	–	27.7.	2 h	1010	–	–	2.8.	1	1598	–	–
24.7.	2	1918	–	–	–	–	28.7.	1	957	–	–	4.8.	3	1442	–	–
28.7.	6	1580	–	–	–	–	31.7.	4	892	–	–	8.8.	7	1599	–	–
31.7.	9	1316	–	–	–	–	3.8.	7	900	–	–	16.8.	14	1425	–	–
8.8.	17	1566	–	–	–	–	6.8.	10	888	–	–	23.8.	22	1151	1	2021
13.8.	23	924	1	2508	–	–	11.8.	15	878	–	–	30.8.	29	1071	8	2148
14.8.	24	843	2	1557	–	–	14.8.	–	–	2 h	1175	6.9.	36	1100	15	2396
18.8.	28	746	6	2091	–	–	17.8.	21	744	3	1027	10.9.	40	1281	19	1698
25.8.	35	835	13	1686	–	–	24.8.	28	711	10	1269	–	–	–	–	–
28.8.	38	726	16	1115	1	2267	28.8.	32	479	14	1094	–	–	–	–	–
1.9.	42	665	20	823	5	2637	–	–	–	–	–	–	–	–	–	–
5.9.	46	694	24	770	9	1814	–	–	–	–	–	–	–	–	–	–

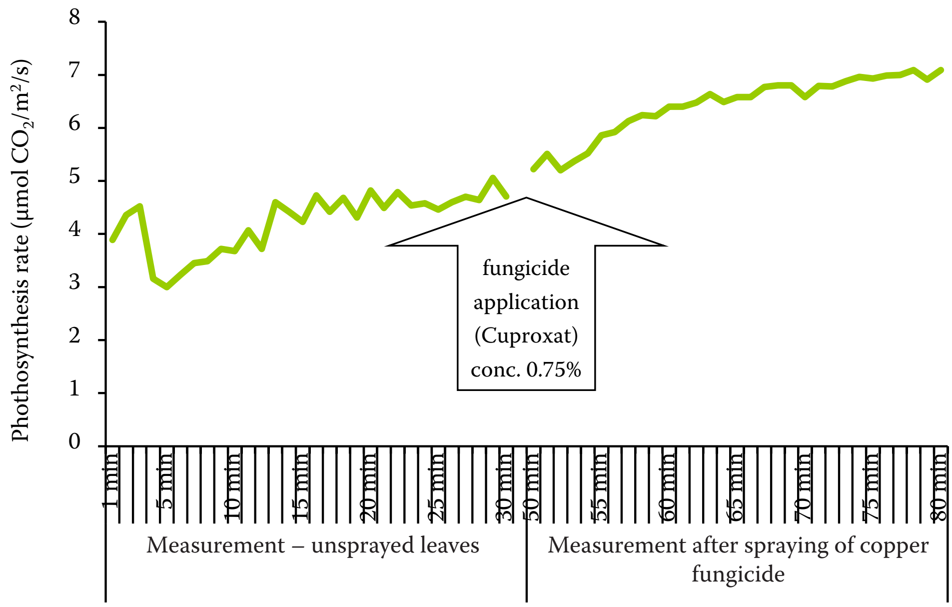

Figure 1. The course of photosynthesis rates of hop plants before and after application of copper fungicide in 2010 (presented rates are current values at the measuring interval)

mediately after the third copper application. Final copper content in cones after all treatment was in the interval of 323 mg/kg in 2010 and 471 mg/kg in 2008. The amount of copper on hop leaves is 2–5 times higher compared to cones. It is given by lower ratio surface/weight, which is 15–20 cm^2/g for cones and 45–60 cm^2/g for leaves. The experiments were carried out in the period when development of leaf area was finished. On the contrary experiment period of hop cones comprised the whole development cycle from the stage of flowering to the harvest ripeness. Application of 5 kg copper per one hectare of vigorous growth of Agnus increases content of copper in hop cones by 300 mg/kg at the ripening period. The same amount of copper increases its content on hop leaves by 1000 mg/kg at least. Tight correlation between the amount of copper applied and its content in hops does not exist. Time series of the copper content on leaves and in cones show perceptible, though irregular, decreasing trend. It can be explained by gradual increase of hop cones size from the stage of flowering at the last decade of July to the mature size in the last decade of August. The same trend on hop leaves is probably caused by washing off, or dissolving of copper compounds by atmospheric water (rain, dew). Small copper demands of plants under the level of 20–25 mg/kg confirm contents of copper in untreated cones in 2009 (Table 3) and organic hops from 2010 crop harvest (22 mg/kg, Table 5). Copper contents found in hops from territories with no downy mildew occurence (China, India, South Africa, Argentina) confirm that natural copper contents in hops are below 20 mg/kg (Krofta

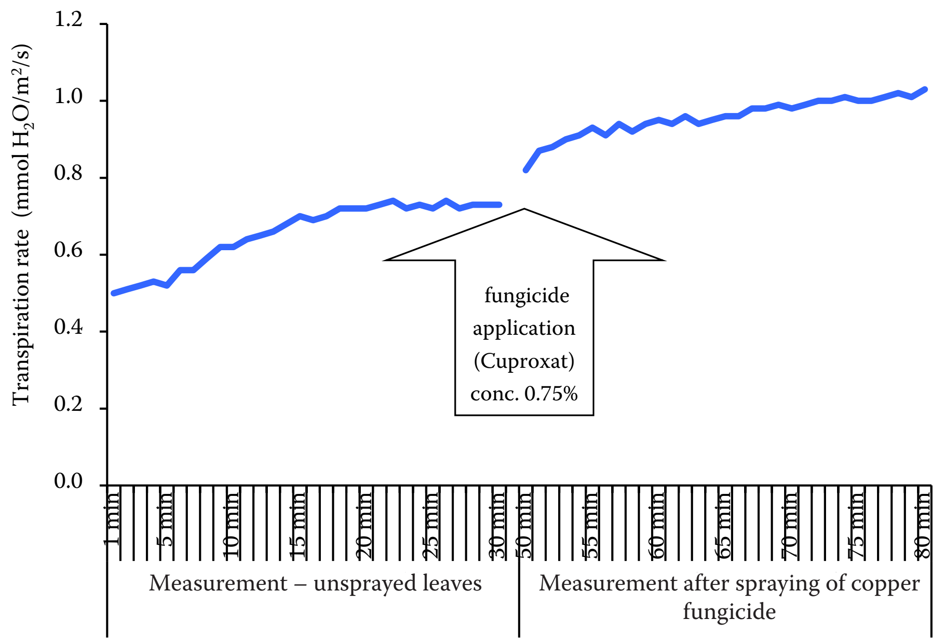

Figure 2. The course of transpiration rates of hop plants before and after application of copper fungicide in 2010 (presented rates are current values at the measuring interval)

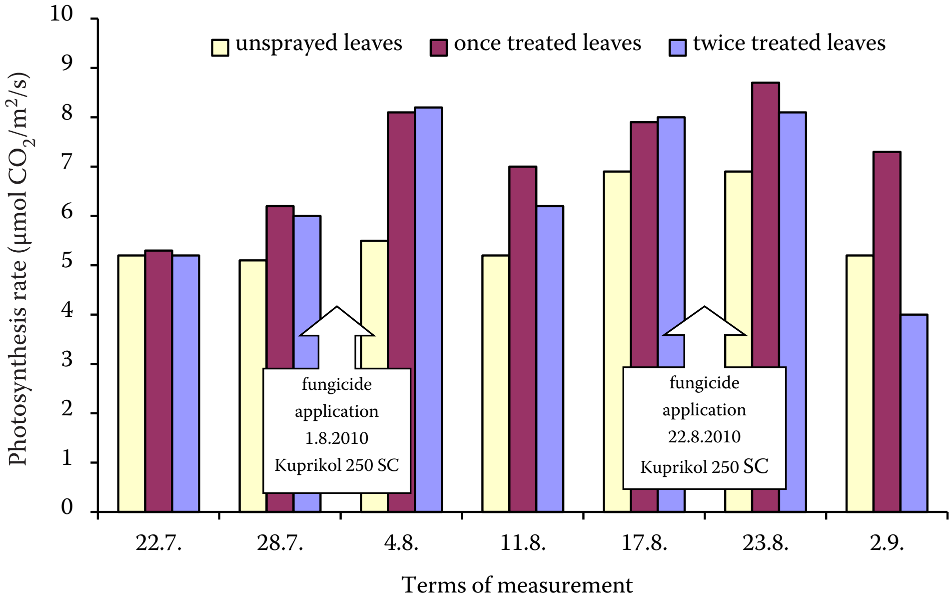

Figure 3. Photosynthesis rates of hop plants in the course of 2010 vegetation season (presented rates are average values from 5 hop plants)

1994). High contents of copper on hop leaves should not be underestimated. Copper from leaves is brought into harvested hops in the form of biological admixtures. Potential contribution of biological admixtures shoud not exceed 60 mg/kg if hops contain up to 3% of admixtures (leaves) and copper contents on hop leaves is max. 2000 mg/kg. Contents of elementary copper in commercial samples of Czech, German and Polish hops from the 2009 and 2010 crop harvest are shown in Table 5. Most of values in Czech hops are in the interval of 20–300 mg/kg. The highest determined value in Premiant variety (633 mg/kg) is still far from the limit 1000 mg/kg valid for EU countries. Comparable data were obtained in Czech hops from the crop harvests 1990 and 1992 (Krofta 1995). Individual values were mostly in the range of 250–350 mg/kg and just rarely contents above 1000 mg/kg were determined. Extremely high contents of copper in hops can be caused by technology mistakes – insufficient homogenisation of powdery preparatives before application. Most of values in German hops are in the interval of 100–400 mg/kg. There are also two limit values, high content in Taurus 807 mg/kg and low value of 19 mg/kg (Hallertauer Tradition) at the level of natural background as well. Copper contents in Polish hops are substantialy lower. Hop growing region is located nearby of Pulawy-Lublin in south-east Poland with different climate and lower infection pressure of fungal diseases.

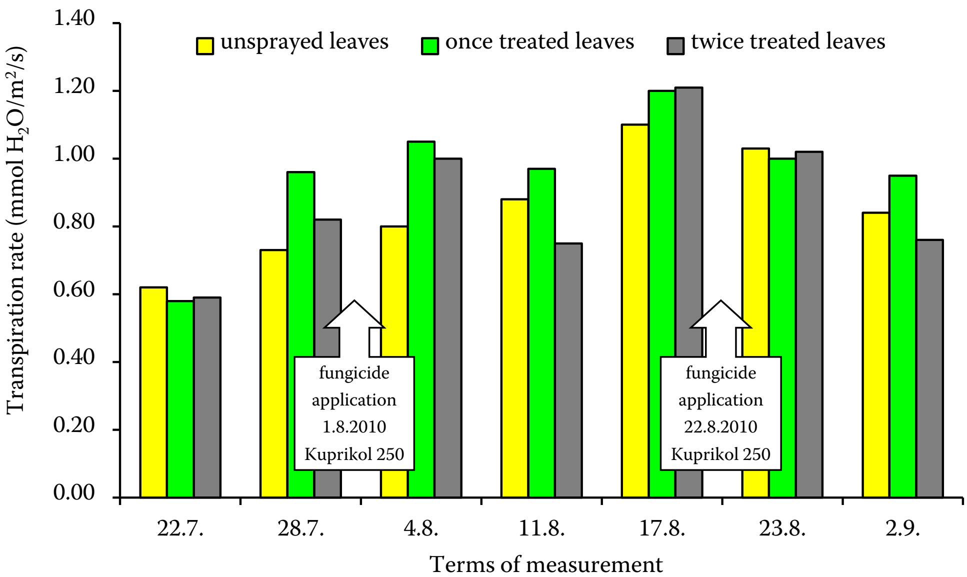

Figure 4. Transpiration rates of hop plants in the course of 2010 vegetation season (presented rates are average values from 5 hop plants)

Table 5. Copper contents in raw hops of Czech, German and Polish varieties, crops 2009, 2010

Variety	Cu content (mg/kg)	Variety	Cu content (mg/kg)
Czech Republic 2010			
Agnus	106	Saaz	148
Agnus	257	Saaz	270
Premiant	74	Saaz	214
Premiant	166	Saaz	145
Premiant	633	Saaz	179
Premiant	278	Saaz	99
Sládek	234	Saaz	192
Sládek	439	Saaz-organic	22
Germany 2010			
Hall. Tradition	19	Herkules	328
Northern Brewer	206	Taurus	807
Perle	170	Spalter Select	249
Saphir	344	Opal	421
Poland 2009			
Sybilla	44	Magnum	30
Lomik	33	Lubelski	153

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