DE GRUYTER
OPEN

Agriculture (Poľnohospodárstvo), 62, 2016 (1): 1−9

Original paper DOI: 10.1515/agri-2016-0001

THE EFFECTS OF SOIL MANAGEMENT PRACTICES ON SOIL ORGANIC MATTER CHANGES WITHIN A PRODUCTIVE VINEYARD IN THE NITRA VITICULTURE AREA (SLOVAKIA)

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ŠIMANSKÝ, V. – POLLÁKOVÁ, N.: The effects of soil management practices on soil organic matter changes within a productive vineyard in the Nitra viticulture area (Slovakia). Agriculture (Poľnohospodárstvo), vol. *62*, 2016, no. 1, p. 1–9.

Since understanding soil organic matter (SOM) content and quality is very important, in the present study we evaluated parameters of SOM including: carbon lability (L_C), lability index (LI), carbon pool index (CPI) and carbon management index (CMI) in the soil as well as in the water-stable aggregates (WSA) under different soil management practices in a commercial vineyard (established on Rendzic Leptosol in the Nitra viticulture area, Slovakia). Soil samples were taken in spring during the years 2008–2015 from the following treatments: G (grass, control), T (tillage and intensive cultivation), T+FYM (tillage + farmyard manure), G+NPK3 (grass + 3rd intensity of fertilisation for vineyards), and G+NPK1 (grass + 1st intensity of fertilisation for vineyards). The highest LI values in soil were found for the G+NPK3 and T+FYM fertilised treatments and the lowest for the unfertilised intensively tilled treatments. The CPI in the soil increased as follows: T < G+NPK3 < T+FYM < G+NPK1. The highest accumulation of carbon as well as decomposable organic matter occurred in G+NPK1 compared to other fertilised treatments, while intensive tillage caused a decrease. On average, the values of LI in WSA increased in the sequence G+NPK1 < T+FYM < G+NPK3 < T. Our results showed that the greatest SOM vulnerability to degradation was observed in the WSA under T treatment, and the greatest values of CPI in WSA were detected as a result of fertiliser application in 3rd intensity for vineyards and farmyard manure application.

Key words: index lability, carbon pool index, carbon management index, fertilisation, soil tillage, vineyard

SOM represents a polyfunctional, uneven-aged, multicomponent continuum of destroyed plant residues, root exudates, microbial biomass, biomolecules, and humic substances. It has lifetimes varying from several hours or days through to millennia (Schepaschenko *et al.* 2013; Semenov *et al.* 2013). SOM content in the soils is influenced by several factors, such as climate, clay content and mineralogy and soil management techniques etc. (Stevenson 1982; Lugato & Berti 2008). Organic carbon content in the soil (SOC) is one of the qualitative parameters of the soil humus regime and long has been recognized as a key component of soil quality (Reeves 1997). SOC can be divided into labile and recalcitrant fractions based on the relative susceptibility to biological decomposition (McLauchlan & Hobbie 2004; Belay-Tedla *et al.* 2009). Labile SOC pools such as water-extractable organic C, hot water-soluble organic C, potassium permanganate oxidizable organic C, and organic C fractions of different oxidizability are considered to respond to agricultural management more rapidly than total organic C (Blair *et al.* 1995; Benbi *et al.* 2012). As such labile fractions of SOM are used as sensitive indicators for soil management and land use induced changes in soil quality (Kolář *et al.* 2011; Benbi

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et al. 2015; Shang *et al.* 2016). Small changes in total SOM are difficult to detect because of its high background levels and natural soil variability. For this reason Blair *et al.* (1995) recommended the use of the carbon lability (L_C), lability index (LI), carbon pool index (CPI) and carbon management index (CMI) for the determination of smaller changes and changes over a short time period. These parameters have been rather quickly adopted and used for the evaluation of SOM changes (Szombathová 1999; 2010). However, data about L_C, LI, CMI and CPI in individual size fractions of water-stable aggregates are very rare.

In this study we evaluated the effect of different soil management practices on L_C, LI, CPI and CMI parameters in (i) the soil and (ii) the water-stable aggregates. Finally, we investigated relationships between these parameters within both the soil and water-stable aggregates.

MATERIAL AND METHODS

The study was conducted at Dražovce (48°21’6.16”N; 18°3’37.33”E), a village located near Nitra city in the west of Slovakia. The area (vineyard) is located under the south-west side of the Tribeč Mountain. In the 11th century, the southern slopes of the Zobor hills were deforested and vineyards were planted. Today the locality is used as a horticulture area and for growing wines. The climate is temperate with a mean annual rainfall of 550 mm and the mean annual temperature ≥ 10°C. The soil was classified as Rendzic Leptosol (WRB 2006) with a sandy loam texture developed on limestone and dolomite. Characteristics of the topsoil (0–30 cm) before the experiment in 2000 are presented in Table 1.

Before vineyard establishment the locality was abandoned. In the year 2000, the vines (*Vitis vinifera* L. cv. Chardonnay) had been planted and up to the year 2003 the vineyard was intensively cultivated in and between rows of the vine (mechanical removal of weeds). In 2003, a variety of grasses in following ratio *Lolium perenne* 50% + *Poa pratensis* 20% + *Festuca rubra commutata* 25% + *Trifolium repens* 5% were sown in and between rows of the vine. In the year 2006, the experimentation of different soil management practices in a productive vineyard was initiated. This experimental design had been previously described by Šimanský & Polláková (2014). Briefly, the treatments consisted of (1). G: as a control (in the rows and between vines rows, a mixture of the grass were sown), (2). T: tillage (in autumn tilth to a depth of 25 cm and intensive cultivation between vine rows during the growing season), (3). T+FYM: tillage + farmyard manure (ploughed farmyard manure at a dose of 40 t/ha in autumn 2005, 2009 and 2012), (4). G+NPK3: doses of NPK fertilisers in 3rd intensity for vineyards, this means 120 kg/ha of N, 55 kg/ha of P and 195 kg/ha of K kg/ha (Fecenko & Ložek 2000). The dose of nutrients was divided: 2/3 applied into the soil in the spring (bud burst) and 1/3 at flowering. The grass was sown in and between the vine rows. (5). G+NPK1: doses of NPK fertilisers in 1st intensity for vineyards, this means 80 kg/ha of N, 35 kg/ha of P and 135 kg/ha of K (Fecenko & Ložek 2000). The dose of nutrients was divided: 1/2 applied into the soil in the spring (bud burst) and 1/2 at flowering. The grass was sown in and between the vine rows.

Sampling was done in the spring throughout the years 2008–2015. Soil samples were collected (0–20 cm layer) from 4 random locations within each treatment of different vineyard soil management practices. Soil samples were then mixed together to form an average sample for each treatment. Samples were air-dried. Then, each of the samples was divided and one half of them were sieved through a 2 mm sieve for chemical analyses and the second half of samples were used for the determination of water-stable aggregates (WSA).

Table 1

Characteristics of a Rendzic Leptosol at Nitra-Dražovce in the year 2000

Soil properties	Means and standard deviation
Rock fragments [%]	8±1.6
Clay [g/kg]	101±12
Silt [g/kg]	330±18
Sand [g/kg]	569±23
Organic carbon [g/kg]	17.0±1.6
Base saturation [%]	99.3±0.01
pH (in 1 mol/dm^3 KCl)	7.18±0.08

Seven aggregate-size fractions (>5, 5–3, 3–2, 2–1, 1–0.5, 0.5–0.25 and <0.25 mm) were separated by the wet-sieving of the soil through the series of six sieves using the Baksheev method. The method for aggregate separation was adopted from Vadjunina and Korchagina (1986). In soil samples as well as in size fractions of WSA, the soil organic carbon (C_{org}) and labile carbon (C_L) contents were determined by Tyurin (Dziadowiec & Gonet 1999) and by Loginow (Loginow *et al.* 1987), respectively. On the base of determined C_{org} and C_L we calculated the following parameters of SOM: carbon lability (L_C), lability index (LI), carbon pool index (CPI) and the carbon management index (CMI), as suggested Blair *et al.* (1995). In this research, the control (G) treatment was the reference and different soil management practices (T, T+FYM, G+NPK3 and G+NPK1) were used as treatments.

Analysis of variance for SOM parameters were performed using Statgraphics Centurion XV.I statistical software (Statpoint Technologies, Inc., USA). The difference between the treatments was examined by one-way analysis of variance (ANOVA) and the *LSD* test ($P < 0.05$) was used for means comparison. Correlation analyses were used to assess the relationship between L_C, LI, CPI and CMI in the soil and the same parameters of SOM in individual size fractions of water-stable aggregates.

RESULTS AND DISCUSSION

SOM in soil

The values of carbon lability (L_C) were not affected by soil management practices, however differences between treatments were observed. The content of L_C increased on average in the following order: G+NPK1 = T < G < T+FYM < G+NPK3. We also evaluated the effect of different soil management practices on changes in SOM parameters such as: LI, CPI and CMI, which are used for determination of smaller changes and changes over a short time period (Blair *et al.* 1995; Szombathová 1999; Vieira *et al.* 2007). Higher values of L_C and LI indicated that SOM was rapidly degradable by micro-organisms, otherwise, lower values of LI indicated SOM had greater stability and resistance to microbial degradation. The highest LI values were found for the G+NPK3, T+FYM and G+NPK1 fertilised treatments and the lowest for the unfertilised, intensively tilled treatments (Table 2). Thus, higher doses of mineral fertilisers as well as organic amendment increased the amount of less stable forms of SOM, mainly by FYM addition, as well as by the promotion of root exudates excretion and the amount of grasses' residues, or through the decay of stable SOM due to high doses of NPK application (G+NPK3). Our findings are in agreement with Fröberg *et al.* (2013), Tong *et al.* (2014), who reported the impacts of mineral fertilisers and effect of manure on mineralization of SOM. Generally,

Table 2

Analyses of variance of soil organic matter parameters

Parameters	Soil management				
	G	T	T+FYM	G+NPK3	G+NPK1
L_C	0.150^a	0.149^a	0.166^a	0.176^a	0.149^a
LI	–	0.831^a	1.104^b	1.184^b	1.073^{ab}
CPI	–	0.807^a	1.014^b	0.956^b	1.073^b
CMI	–	70.800^a	113.700^b	116.000^b	116.100^b

G – control; T – tillage; T+FYM – tillage+farmyard manure; G+NPK3 – doses of NPK fertilisers in 3rd intensity for vineyards; G+NPK1 – doses of NPK fertilisers in 1st intensity for vineyards

L_C – carbon lability; LI – lability index; CPI – carbon pool index; CMI – carbon management index

Different letters between columns (a, b) indicate that treatment means are significantly different at $P < 0.05$ according to *LSD* multiple-range test labile SOM is highly susceptible to mineralization. Our results non-significantly confirmed this fact by low value of CPI in G+NPK3 treatment. Conversely, intensive cultivation (T treatment) was responsible for microbial decomposition of SOM, since aeration caused by cultivation stimulated decomposition and the subsequent mineralization of both labile and also later stable forms of SOM (Prasad *et al.* 2016), which resulted in an overall decrease of SOM quantity. Subsequently we found then that the T treatment contained the lowest stock of C$_{org}$ (13.0 g/kg), and also, significantly, the lowest value of CPI (0.807). When we expressed LI from C$_{org}$ (C$_{org}$ varied in different treatments: G = 17.4 g/kg, T = 13.0 g/kg,

Table 3

Analysis of variance of organic and labile carbon contents in size fractions of water-stable aggregates

Parameters	Size fraction of water-stable aggregates in mm		Treatments				
			G	T	T+FYM	G+NPK3	G+NPK1
Lc	WSA$_{mi}$	<0.25	0.147^{a}	0.197^{b}	0.176^{ab}	0.197^{b}	0.154^{ab}
	WSA$_{ma}$	0.25–0.5	0.143^{a}	0.183^{a}	0.177^{a}	0.164^{a}	0.164^{a}
		0.5–1.0	0.140^{a}	0.159^{a}	0.151^{a}	0.176^{a}	0.173^{a}
		1.0–2.0	0.130^{a}	0.192^{b}	0.159^{a}	0.149^{a}	0.130^{a}
		2.0–3.0	0.132^{a}	0.166^{a}	0.150^{a}	0.147^{a}	0.146^{a}
		3.0–5.0	0.139^{ab}	0.159^{ab}	0.152^{ab}	0.168^{b}	0.134^{a}
		>5.0	0.128^{a}	0.169^{b}	0.162^{ab}	0.162^{ab}	0.133^{ab}
LI	WSA$_{mi}$	<0.25	–	1.372^{a}	1.232^{a}	1.369^{a}	1.077^{a}
	WSA$_{ma}$	0.25–0.5	–	1.295^{a}	1.262^{a}	1.164^{a}	1.124^{a}
		0.5–1.0	–	1.155^{a}	1.099^{a}	1.316^{a}	1.254^{a}
		1.0–2.0	–	1.556^{b}	1.293^{ab}	1.178^{ab}	1.061^{a}
		2.0–3.0	–	1.292^{a}	1.159^{a}	1.147^{a}	1.127^{a}
		3.0–5.0	–	1.165^{ab}	1.110^{a}	1.274^{b}	0.989^{a}
		>5.0	–	1.453^{a}	1.354^{a}	1.329^{a}	1.109^{a}
CPI	WSA$_{mi}$	<0.25	–	0.824^{a}	1.038^{b}	1.061^{b}	1.053^{b}
	WSA$_{ma}$	0.25–0.5	–	0.915^{a}	1.165^{b}	1.087^{ab}	1.098^{ab}
		0.5–1.0	–	0.891^{a}	1.127^{b}	1.017^{ab}	1.035^{ab}
		1.0–2.0	–	0.955^{a}	1.173^{b}	1.056^{ab}	1.062^{ab}
		2.0–3.0	–	1.019^{a}	1.209^{b}	1.167^{ab}	1.094^{ab}
		3.0–5.0	–	1.098^{a}	1.334^{b}	1.191^{ab}	1.126^{a}
		>5.0	–	1.087^{a}	1.439^{b}	1.134^{a}	1.030^{a}
CMI	WSA$_{mi}$	<0.25	–	117.2^{a}	131.9^{a}	143.4^{a}	112.8^{a}
	WSA$_{ma}$	0.25–0.5	–	119.7^{a}	146.6^{a}	126.3^{a}	118.8^{a}
		0.5–1.0	–	99.6^{a}	122.8^{a}	132.9^{a}	122.6^{a}
		1.0–2.0	–	146.6^{a}	149.7^{a}	121.5^{a}	119.1^{a}
		2.0–3.0	–	122.9^{a}	140.9^{a}	133.5^{a}	117.2^{a}
		3.0–5.0	–	131.2^{a}	148.7^{a}	152.9^{a}	109.7^{a}
		>5.0	–	171.9^{a}	197.5^{a}	151.5^{a}	117.0^{a}

G – control; T – tillage; T+FYM – tillage+farmyard manure; G+NPK3 – doses of NPK fertilisers in 3rd intensity for vineyards; G+NPK1 – doses of NPK fertilisers in 1st intensity for vineyards
L$_{C}$ – carbon lability; LI – lability index; CPI – carbon pool index; CMI – carbon management index
WSA$_{mi}$ – water-stable micro-aggregates; WSA$_{ma}$ – water-stable macroaggregates
Different letters between columns (a, b) indicate that treatment means are significantly different at $P < 0.05$ according to *LSD* multiple-range test

T+FYM = 17.4 g/kg, G+NPK3 = 16.8 g/kg, in G+NPK1 = 18.4 g/kg), the most intense mineralization was observed in G+NPK3 < T+FYM < T < G+NPK1. Using Blair *et al.* (1995) and Conteh *et al.* (1999) recommendation of the use of CPI for determination of SOM content, we found the lower the CPI value is, the more soil degradation is intensified in terms of reduction of soil organic matter content. Soil CPI increased in the following order: T < G+NPK3 < T+FYM < G+NPK1. However, CPI was lower in T treatment by 18%, 26% and 33% than in G+NPK3, T+FYM and G+NPK1, respectively. We also calculated the values of CMI in the soil to examine the impact of soil management practices. Usually, lower values of CMI indicate more intensive changes in the content of organic matter due to soil management practices and more carbon released from the soil stock (Blair *et al.* 1995). In our study, when considering CMI indices, the most intense change was caused as a result of intensive cultivation. The highest accumulation of carbon as well as decomposable organic matter occurred in G+NPK1 (Table 2), while intensive tillage caused decreases in not only SOM content, but also the percentage of its labile forms, since these were quickly mineralized due to cultivation.

SOM in water-stable aggregates

Soil management practices in the vineyard had a statistically significant influence on L_C in WSA. The largest values of L_C in water-stable micro-aggregates (WSA_{mi}) were found for the T, G+NPK3, then T+FYM and G+NPK1, whilst the smallest influence was seen for the G. The highest statistically significant difference of the L_C in WSA_{mi} was observed between the control and treatment with added fertilisers in 3rd intensity for vineyards as well as tillage treatment. Carbon lability indices in greater sized (water-stable macro-aggregates) WSA_{ma} (> 3mm) copied this trend as was also seen in L_C in WSA_{mi} of the investigated soil treatments. In size fractions of WSA_{ma} 1–2 mm and >5 mm the highest differences were observed between the control and tilled treatment. In all treatments, except G+NPK1, higher values of L_C were determined in WSA_{mi} than WSA_{ma}, which indicated higher proportions of labile carbon were in micro-aggregates. This means that micro-aggregates were more sensitive to the microbial decomposition than macro-aggregates. This is surprising because previous literature reports the opposite finding. Peth *et al.* (2008) and Kögel-Knabner *et al.* (2008) reported that SOM inside of micro-aggregates is more stabile due to better physical protection and physico-chemical protection. On average, the values of LI in WSA increased in the sequence G+NPK1 (1.11±0.08) < T+FYM (1.22±0.10) < G+NPK3 (1.30±0.11) < T (1.33±0.15). The largest differences (statistically significant) were found between treatments T and G+NPK1 in fractions of WSA_{ma} 1–2 mm and between the G+NPK3 and G+NPK1 in fractions of WSA_{ma} 3–5 mm. Lobe *et al.* (2001) reported that the largest content of total carbon (60–90%) is found in small macro-aggregates and that micro-aggregates up to 40% may decrease carbon supply as a result of cultivation compared with meadows. Our results showed that the greatest vulnerability to degradation of organic matter was observed in the micro-aggregates (the greatest Lc and LI values) and also macro-aggregates (the greatest average L_C and LI values) under intense cultivation of vine rows, which indirectly confirmed the findings of Lobe *et al.* (2001). The highest values of the CPI in WSA_{ma} were detected as a result of farmyard manure application (Table 3). The results point to the fact that SOM is degraded not only in the soil but also in the WSA, especially due to intensive soil cultivation, which confirmed findings of several studies (Khorramdel *et al.* 2013; Abdollahi *et al.* 2014), and also as a result of the application of high doses of fertilisers to the soil (Yang *et al.* 2011). Results obtained in this study showed, that the greatest enrichment in C in WSA occurred in the T+FYM treatment, the depletion in C in T treatments, whereas in G+NPK1 and G+NPK3 treatments the values were almost the same. In T treatment, the average CPI in macro-aggregates was lower than 1, what means a decreasing trend of organic carbon. In T treatment in addition to macro-aggregates, the CPI value was not lower than 1 also in micro-aggregates, which means that the microbial decomposition of organic matter occurred at the level of micro-aggregates, which may gradually result in the collapse of soil structure. Moreover, the lability of organic matter (L_C) was the greatest just in T treatment (Table 3), indicating greater susceptibility of organic matter to decomposition. Although organic matter lability

(L_C) means the biodegradability of labile organic matter forms, the cultivation without organic and mineral fertilisers added, considerably accelerated the decrease of C_{org} – when in control treatment C_{org} was 17.4 g/kg, and in T treatment 13.0 g/kg. Generally, labile (active) forms of organic matter are precursors of the stable (passive, slow) forms of SOM. When soil lacks labile organic matter, soil micro-organisms gradually use as a source of nutrients and energy more stable forms of SOM, and the result is a slow, gradual decrease of organic matter in the soil (Brady & Weil 1999). Surprising finding was revealed that the largest changes in stocks of SOM in all soil management practices occurred in WSA_{mi}

Table 4

Correlation between SOM parameters in soil and water-stable aggregates

Soil management	SOM parameters in soil	Size fractions of water-stable aggregates						
		>5	5–3	3–2	2–1	1–0.5	0.5–0.25	<0.25
Together		L_C						
	L_C	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		LI						
	LI	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		CPI						
	CPI	0.398^{+}	n.s.	0.379^{+}	n.s.	0.384^{+}	n.s.	0.358^{+}
		CMI						
	CMI	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		L_C						
G	L_C	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
T		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
T+FYM		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G+NPK3		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G+NPK1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		LI						
T	LI	n.s.	n.s.	n.s.	n.s.	n.s.	0.739^{+}	n.s.
T+FYM		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G+NPK3		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G+NPK1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		CPI						
T	CPI	0.806^{+}	0.750^{+}	n.s.	n.s.	n.s.	n.s.	n.s.
T+FYM		n.s.	0.734^{+}	n.s.	n.s.	n.s.	n.s.	n.s.
G+NPK3		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G+NPK1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		CMI						
T	CMI	n.s.	n.s.	n.s.	n.s.	0.717^{+}	n.s.	n.s.
T+FYM		0.723^{+}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G+NPK3		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G+NPK1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

$^{+}P \leq 0.05$; n.s. – non-significant
G – control; T – tillage; T+FYM – tillage+farmyard manure; G+NPK3 – doses of NPK fertilisers in 3rd intensity for vineyards; G+NPK1 – doses of NPK fertilisers in 1st intensity for vineyards
L_C – carbon lability; LI – lability index; CPI – carbon pool index; CMI – carbon management index

compared to WSA_{ma}. For example, Six *et al.* (2004) reported that macro-aggregates are less stable due to intensive soil cultivation and therefore break-up into micro-aggregates. Thus, significant changes in the carbon content, particularly in the largest fractions WSA_{ma} recorded by Gale *et al.* (2000), are not consistent with our findings (Table 3). In the WSA we also calculated CMI indices depending on the soil management practices in vineyard. Overall, smaller CMI values, indicating minor changes in the content and quality of organic matter due to land management, were recorded more in WSA_{mi} than WSA_{ma} (except G+NPK3). The lowest value of CMI in WSA_{mi} was determined in G+NPK1 and T treatments (Table 3). One-way ANOVA analysis did not confirm significant differences between treatments in contents of WSA_{ma}. The largest accumulation of SOM was detected in the size fraction of WSA_{ma} > 5 mm due to application of farmyard manure and application of fertilisers in 3rd intensity for vineyards, but also due to intensive cultivation of vine rows. In G+NPK1, the highest accumulation of SOM was observed in size fraction of WSA_{ma} 5–3 mm.

Correlations between SOM parameters in soil and in WSA

Correlation coefficients between SOM parameters in soil and in WSA are shown in Table 4. When the L_C and LI values were assessed together regardless of soil management practices, no correlation was recorded. However, the value of LI in soil positively correlated with LI in WSA_{ma} 0.5–0.25 mm, but only under T treatment. This means that intensive cultivation between vine rows can increase the lability of carbon in smaller macro-aggregates. Statistically significant positive correlations were observed between CPI in soil and CPI in WSA (together), and this effect was stronger in size fractions of 5–3 mm, 2–1 mm and 0.5–0.25 mm. As the CPI values were assessed with relation to soil management practices, we detected a positive significant correlation between CPI in soil and CPI in WSA_{ma} in size fractions of >5 mm and 5–3 mm under intensive cultivated rows of vine, and in fractions of 5–3 mm in treatments with ploughed farmyard manure (Table 4). Statistically significant positive correlation was observed between CMI in soil and CMI in WSA_{ma} 1–0.5 mm, if the CMI values were assessed together, regardless of soil management practices in the vineyard. Evaluating CMI values in relation to soil management practices, we only detected positive significant correlation between CMI in soil and CMI in WSA_{ma} in size fractions of 1–0.5 mm under T treatment and > 5mm in T+FYM treatment (Table 4).

CONCLUSIONS

This study indicates that the highest accumulation of carbon, as well decomposable organic matter in soil, occurred in treatments with the application of fertilisers in 1^{-st} intensity for vineyards compared other fertilised treatment, while intensive tillage caused the decrease not only of total SOM content, but also its labile forms, which were quickly mineralized due to cultivation. Similarly, the greatest vulnerability of organic matter to degradation was observed in the WSA under T treatment, however, the highest accumulation of SOM in WSA were detected as a result of farmyard manure application. Results further showed that between CPI in soil and WSA there were significant relationships if all soil management practices were assessed together. When soil management practices in a vineyard have been assessed separately, there were clear relationships between CPI in soil and higher size fraction of water-stable macro-aggregates.

Acknowledgements. We thank Matthew Evans (Brackley Northants, England) for help with improving the editorial presentation of this paper. Project supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (No. 1/0604/16).

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Received: February 16, 2016

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 62, 2016 (1): 10−18

DOI: 10.1515/agri-2016-0002 Original paper

EFFECT OF LAND USE CHANGE ON SOIL ORGANIC CARBON

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BARANČÍKOVÁ, G. – MAKOVNÍKOVÁ, J. – HALAS, J.: Effect of land use change on soil organic carbon. Agriculture (Poľnohospodárstvo), vol. *62*, 2016, no. 1, pp. 10–18.

The direction of changes and conversion of soil organic carbon (SOC) is in most current ecosystems influenced by human activity. Soil Science and Conservation Research Institute is responsible for monitoring the agricultural soils in a five- year cycle. One part of the soil monitoring involves the determination of the soil organic carbon (SOC) storage. Further, we followed the conversion of arable land on grassland during more than 20 years of monitoring period at some locations where changes in land use occurred. Ten places on basic network and 2 places on key monitoring localities in which arable land have been converted into grassland were identified. About 50 percent of studied soils converted into permanent grassland were Cambisols. The other converted soil types were Luvic Stagnosol, Stagnic Regosol, Mollic Fluvisol, and Stagnic Luvisol. The results showed that after the third monitoring cycle (2002), increase of SOC was observed in all the localities, with the change in land use. Statistical parameter (t-test) confirmed significant differences between the set of average SOC values before and after the land use conversion. The chemical structure of humic acids (HA) isolated from arable soil and permanent grassland indicated increasing of aliphatic carbon content in grassland HA. More aromatic and stabile were HA isolated from arable soils.

Key words: soil organic carbon, total nitrogen, humic acids, arable land, grassland

The soil organic matter (SOM) and its key component, soil organic carbon (SOC), is one of the most important elements in the soil system. SOC is substantive and energetic basis of all biological soil processes and thus it is the basis of the most productive and nonproductive soil function. SOC is also one of the major criteria in the internationally used soil classification system (Micheli *et al.* 2014). The direction of changes and conversion of SOC is in the most current ecosystems influenced by human activity – land use. This is especially true for agricultural land, where soil organic matter represents more as 95% (pastures, meadows) or almost 100% (arable land) of total organic carbon accumulated in human-amended ecosystems (Stolbovoy & Montanarella 2008). The changes in land use means changes in total stock of soil organic carbon. The soils easily and quickly lose organic carbon when natural soils are converted into agricultural soils as vice versa (changes of arable land to permanent grassland, or meadows, respectively agroforestry). It is estimated that soil cultivation, mainly conversion of pasture into arable land, leads to significant organic carbon loses in the overall balance up to 50Pg (Janzen 2006); conversely land-use change can offer an opportunity for sequestering atmospheric carbon in soils (Janzen 2006). Guo and Gifford (2002), on the basis of meta analysis, show that the conversion of pasture into arable land SOC decreased up to 59%; however, change from crop to pasture can increase the SOC stock (19%). Dawson and Smith (2007) also reported decrease of SOC

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stock by conversion of forest or meadow into arable land, however, converted cropland to grassland can be sequestrated to soil carbon. Smith (2014) notes that the accumulation of organic carbon by grasslands will only occur under optimal management. Generally, it can be concluded that wherever one of the land use decreases the soil organic carbon, the reverse process usually increases it and vice versa (Guo & Gifford 2002). In present time, when climate changes induced a loss of SOC, land use has the great impact on SOC dynamics on the landscape scale (Minasny *et al*. 2014). Changes in land use occurred also in the Slovak agricultural lands and during the last 20 years, it is observed decreasing of arable lands and increasing of grasslands (Statistical Yearbook of the Slovak Republic 1994, 2014). In Slovakia, it has been the regular soil monitoring since 1993 in a five-year cycle. One part of the soil monitoring system is also monitoring of soil organic carbon stock. More than 20 years of monitoring showed the changes in SOC stock, especially after land use conversion.

In this work, we present the changes in content and quality of soil organic matter and total nitrogen on monitoring localities after conversion of arable lands into permanent grasslands.

MATERIAL AND METHODS

Monitoring network, with five-year monitoring cycle, on the selected 10 localities is given in Figure 1. Changes in SOC stock after land use conversion were detected in the following soil types: Haplic Cambisols, Stagnic Cambisols, Luvic Stagnosol, Stagnic Regosol, Mollic Fluvisol, and Stagnic Luvisol (Societas pedologica slovaca 2014). Changes of SOC stock were also found in Haplic Cambisol (HC) and Haplic Stagnosol (HS) after conversion of arable land to grassland on two key monitoring localities (Figure 1).

Soil organic carbon content was determined according to Turin method in Nikitin modification in 1993, 1997, and 2002. In 2007, the total content of C and N was determined using analyser EA (Barančíková *et al*. 2011). For the evaluation of SOC values, > 3% of previous cycle with last one (2007) PTF function was used. The comparison of differences in SOC stock between results of EA and Turin method is published in Barančíková and Makovníková (2015). Total nitrogen (N_{tot}) was determined in 1993, 1997, and 2002 according to the Jodlbauer method (Fiala *et al.* 1999). In 2007, we also determined N_{tot} on C, N by analyser EA (Barančíková *et al.* 2011). Both methods of N_{tot} determination gave the same results. Qualitative parameter such as optical parameter ($Q^4{}_6$) was determined according to Konovovova-Beľčikova method (Barančíková *et al.* 2011).

Isolation of humic acids (HA) was realised according to the IHSS method. The basic parameters of HA chemical structure (elemental analysis and parameters calculated of ^{13}C NMR spectra) were found (Barančíková *et al*. 2011).

Statistical analysis and data evaluation were carried out using the program Statgraphic XV. Centurion.

RESULTS AND DISCUSSION

Land use changes on localities of basic network

Land-use conversion can be found in all of the Slovak regions (Figure 1). It includes different soil types (Table 1), however, mostly Cambisols (50%) were studied. Cambisols are the most widespread soil type in Slovakia (Bielek 2014). They occur mainly on hilly and mountain regions, which in last 20 years were converted from arable land into permanent grasslands (Bielek 2014; Statistical Yearbook of the Slovak Republic 1994, 2014).

All the observed localities were divided into three groups. The first group includes only Cambisols, and the second rest of the soil types (Luvic Stagnosols, Stagnic Regosol, Mollic Fluvisol, and Stagnic Luvisol), and the third group represents all the localities (Table 1). There were three monitoring cycles – the first in 1993, the second in 1997, and the third in 2002. At the beginning of soil monitoring (1993), all of soils were used as arable lands. In the second monitoring cycle (1997), some of them were converted into the grassland. In 2002 (the third monitoring cycle), all of them were used as permanent grassland (Table 1). Moreover, in 2013 (the fourth monitoring cycle), two of them were completely removed from agricultural land resources.

This confirms the trend of agricultural land decreasing in Slovakia in the last five years (Statistical Yearbook of the Slovak Republic 1994, 2014).

In Figure 2, the average values for three soil groups in three monitoring cycles are given. The results show that conversion of arable land into grassland leads to increasing of SOC. This trend is similar on Cambisols (group 1), on group 2, where different soil types are present, and on all the observed localities (group 3). The increase of SOC from crop to pasture is in agreement with literature data (Guo & Gifford 2002).

In determining the differences of SOC between arable land and grassland, we compared the two sets. File arable land (SOC average values of individual locations during their use as arable land) and a set of grasslands (SOC average of the same locations during their use as grasslands). Figure 3 (Box-Whisker plot) shows the significant difference in average between the arable lands and permanent grasslands (test to compare means *t*-test, $t = -2.10014$, the level of significance $\alpha = 0.05$).

It is clear that higher SOC values are in grassland to compare with cropland (Gerzebek *et al*. 2006;

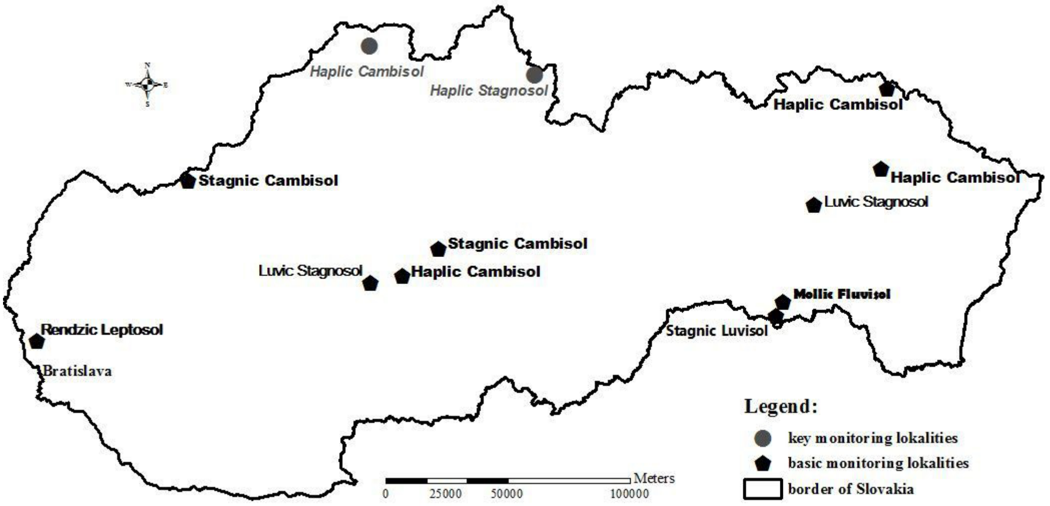


Figure 1. Localities of basic network and key monitoring localities with land-use change

Table 1

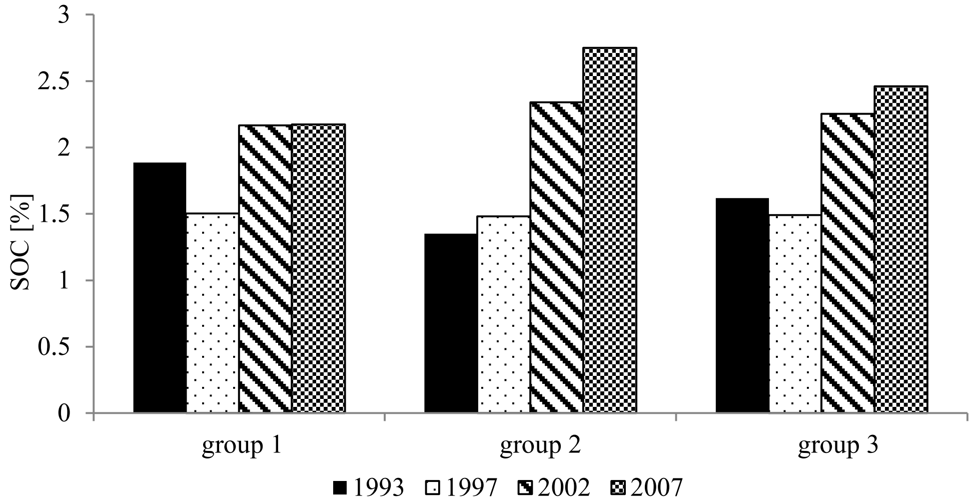
Monitoring localities of basic network with land use change

Group	Locality	Soil type	Land use				
			1993	1997	2002	2007	2013
Group 1	Horná Mičiná	Stagnic Cambisol	AL	G	G	G	G
	Tŕnie	Haplic Cambisol	AL	AL	G	G	G
	Nová Bošáca	Stagnic Cambisol	AL	AL	G	G	G
	Krajná Bystrá	Haplic Cambisol	AL	G	G	G	G
	Turany	Haplic Cambisol	AL	G	G	G	G
Group 2	Záborské	Luvic Stagnosol	AL	AL	G	G	G
	Horné Opatovce	Luvic Stagnosol	AL	G	G	G	0
	Veľká Ida	Mollic Fluvisol	AL	G	G	G	G
	Záhorská Bystrica	Stagnic Regosol	AL	AL	G	G	0
	Nižný Lánec	Stagnic Luvisol	AL	G	G	G	G

AL – arable land; G – grassland; 0 – nonagricultural land

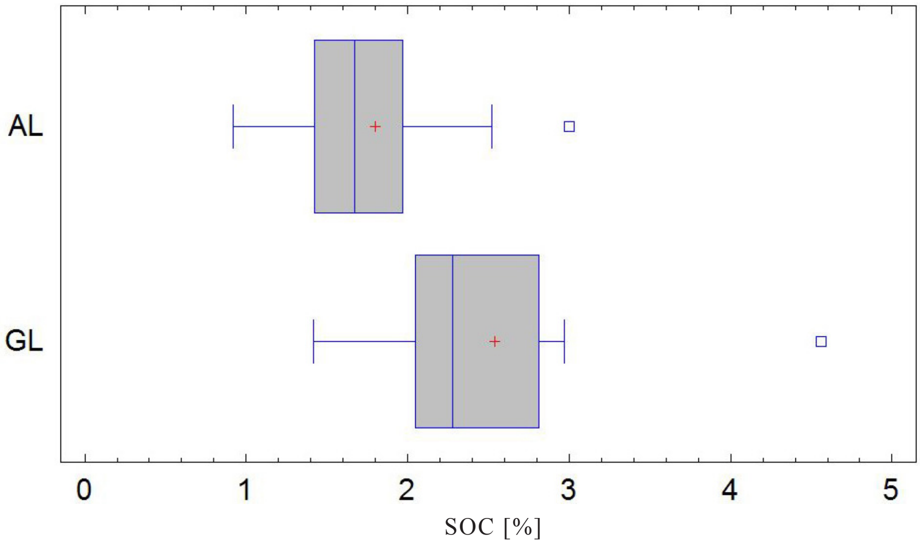
Barančíková 2014; Gelaw *et al*. 2014; Manu *et al.* 2014; Sanford 2014). Generally speaking, higher input of plant and root residues in grassland soils is stabilizing the SOC stock in the top soil. The wrong soil management, mainly intensive agriculture and low input of organic debris, can highly increase the mineralization processes. The last caused decreasing of SOC in the arable land.

Soil organic matter is an important source of basic biogenic elements for plant growth. More than 95% of the most important biogenic elements such as nitrogen and sulfur are bound in SOM (Baldoc & Nelson 1999). In accordance with this statement, the values of total nitrogen are in close relationship with soil organic carbon content. Our results confirmed this fact and the significant linear correlation ($R = 0.85^{++}$,



group 1 – Cambisols; group 2 – Luvic Stagnosol; Stagnic Regosol; Mollic Gleyic Fluvisol, and Stagnic Cutanic Luvisol; group 3 – all soil types
SOC – soil organic carbon

Figure 2. Average values of SOC on localities where the land use change was found



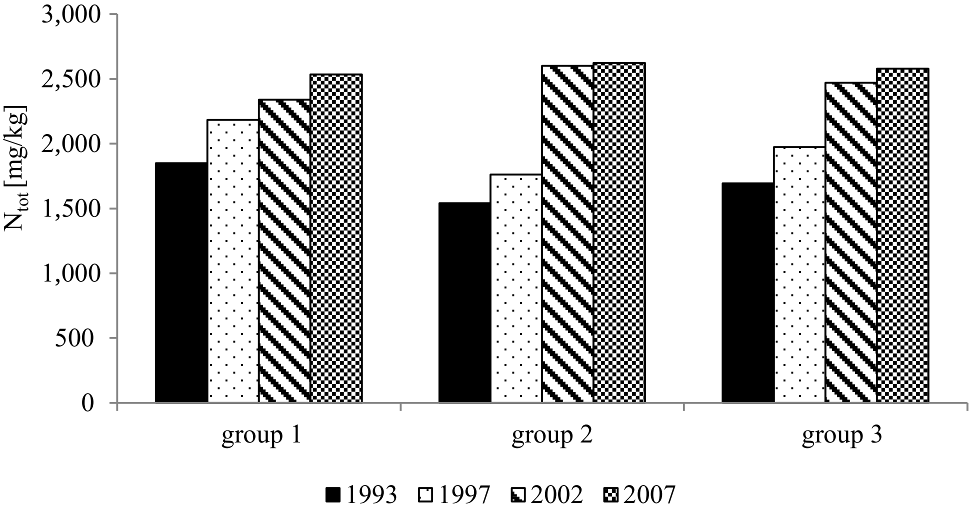
AL – average value of SOC for arable land; GL – average value of SOC for grassland; SOC – soil organic carbon

Figure 3. Box-Whisker plot

n = 10) between N_{tot} and SOC was found. Increasing of total N_{tot} after conversion of arable land was observed (Figure 4).

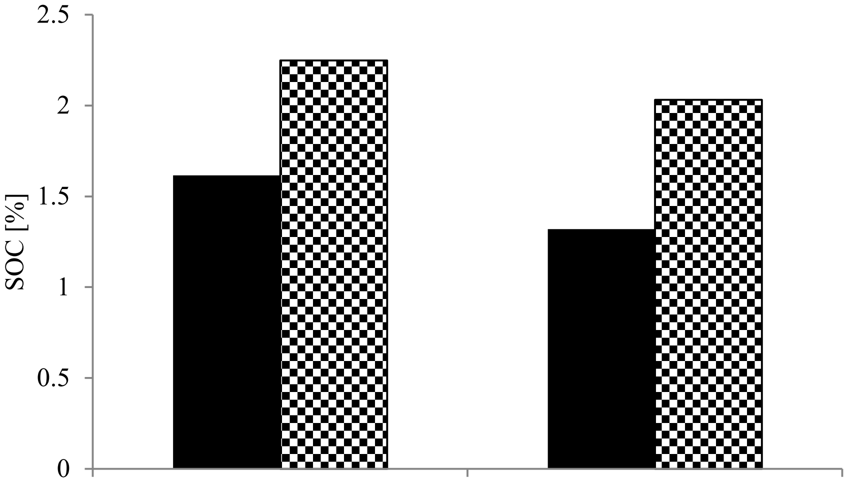
Land use changes on key monitoring localities

Changes of SOC stock were also found in Haplic Cambisol (HC) and Haplic Stagnosol (HS) after conversion of arable land to grassland in two key monitoring localities. These soil types represent the less productive agricultural soils (Bielek 2014). Both of these localities were situated in the north part of Slovakia (Figure 1) in the mountain region. Soil sampling was done here every year. At the beginning of soil monitoring (time period 1994–2001), these localities were used as arable land and later they became permanent grasslands. The comparison



group 1 – Cambisols; group 2 – Luvic Stagnosol; Stagnic Regosol; Mollic Gleyic Fluvisol and Stagnic Cutanic Luvisol; group 3 – all soil types
N_{tot} – total nitrogen

Figure 4. Average values of N_{tot} on localities where the land use change was found



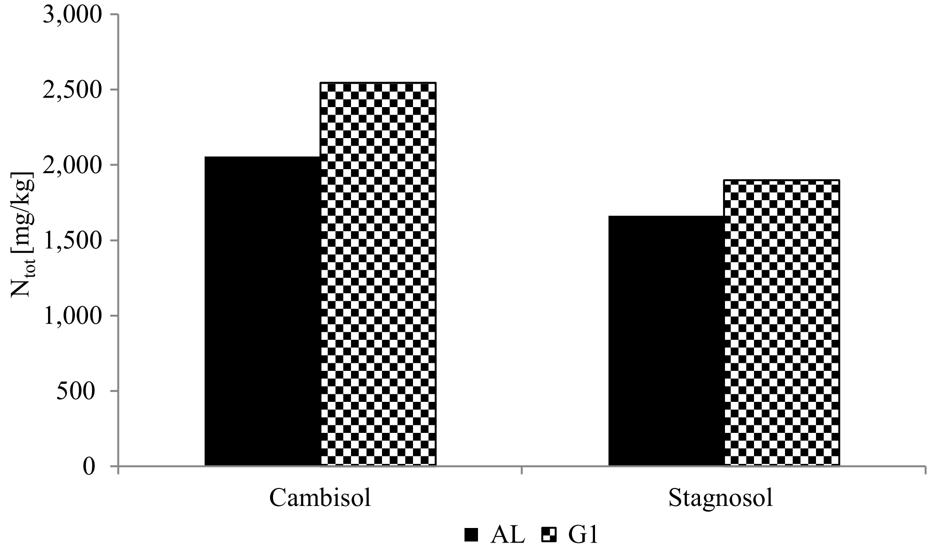
AL – average value of SOC of time period 1994–2001 (arable land); G – average value of SOC of time period 2002–2014 (grassland); SOC – soil organic carbon

Figure 5. Development of SOC on two key monitoring localities during monitoring period

of average SOC values during 1994–2001, when these localities were used as arable land (AL), and during 2002–2014, when these localities were converted into the grassland (G), are given in Figure 5.

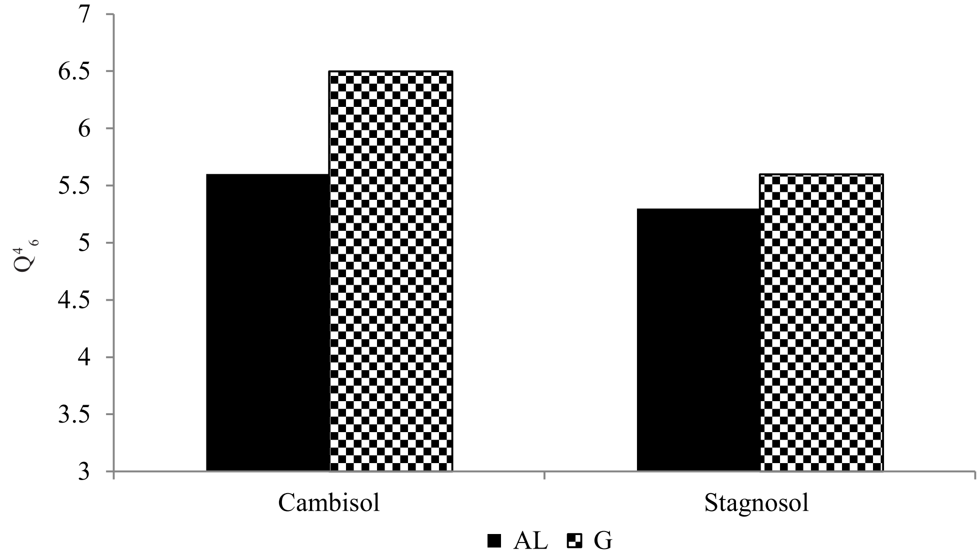
Total nitrogen content is given in Figure 6. Increase of SOC content and N_{tot} content after land conversion was evident.

Qualitative parameters, such as absorption in UV-VIS spectral range and Q^4_6 indexes, were determined. The optical index (Q^4_6) represents the absorbance ratio of humic acid solution at λ = 465 and λ = 665 nm (Barančíková *et al*. 2011). Higher values of this parameter are characteristic of more labile and less mature soil organic matter, and on the con-



AL – average value of N_{tot} of time period 1994–2001 (arable land); G – average value of N_{tot} of time period 2002–2014 (grassland)

Figure 6. Development of N_{tot} on two key monitoring localities during monitoring period



AL – average value of Q^4_6 of time period 1994–2001 (arable land); G – average value of Q^4_6 of time period 2002–2014 (grassland)

Figure 7. Development of Q^4_6 on two key monitoring localities during monitoring period

trary lower values of Q^4_6 are typical of mature and well-humificated soil organic matter. Both observed localities showed higher values of Q^4_6 in grassland soils to compare with arable soil. We can assume that more labile and less mature is SOM of grassland compared with arable soils (Figure 7).

Humic acids were isolated every three years from the key monitoring localities. HA from HC were isolated in 1995, 1998, 2001, 2004, 2007, and 2012. HA from HS were isolated in 1994, 1997, 2000, 2003, 2006, 2009, 2014. Data gathered during 1995–2001 represent HC used as arable soil. Data collected during 2004–2012 represent the same soil used as permanent grassland. Data obtained during 1994–2000 represent HS used as arable soil. Data that were collected during 2003–2014 represent the same soil used as permanent grassland.

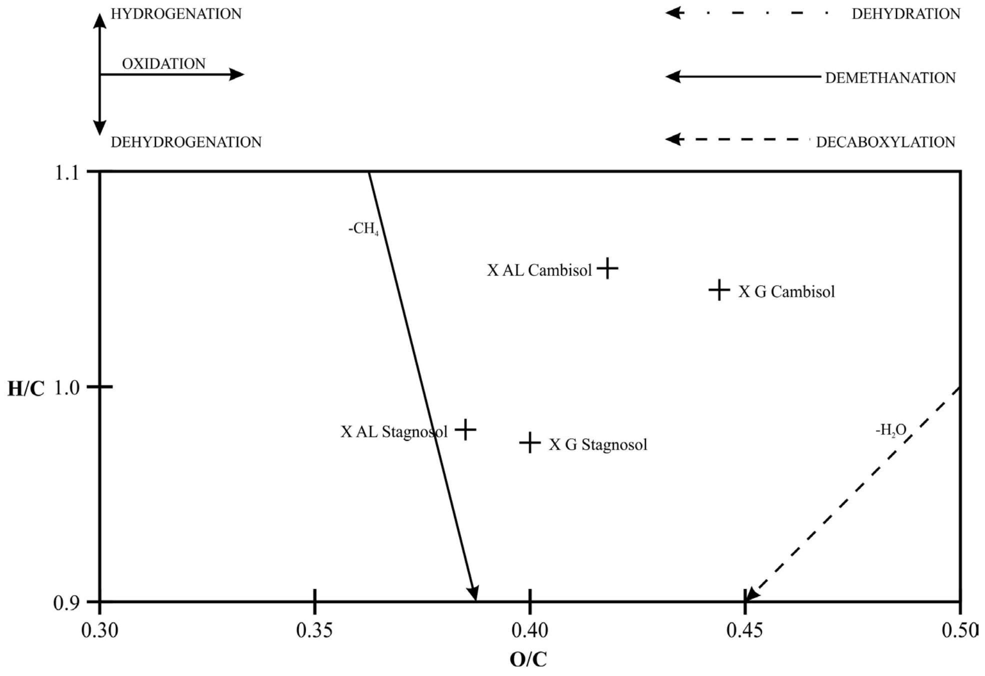
Evaluation of HA structure and elemental composition reflect the dominant characteristic of soil humification. Depending on the HA origin, the carbon content differs. Generally, it represents 35–50% atomic carbon, and 35–45% atomic hydrogen (Than 2003). Higher content of carbon and lower percentage of hydrogen is characteristic for organic materials with a higher humification degree. The results obtained for HA elemental composition in Haplic

Table 2

Elemental composition and calculated parameter of ^{13}C NMR spectra of humic acids

HA	C	H	O	N	C_{alif} [%]	C_{ar} [%]	α [%]
x-AL HS	41.00	40.17	15.82	3.00	46.85	33.81	41.94
x-G HS	40.62	39.54	16.25	3.60	49.51	33.45	40.50
x-AL HC	38.94	41.04	16.29	3.75	50.54	29.42	36.76
x-G HC	38.53	40.25	17.12	4.10	56.85	25.01	30.71

x-AL average value of elemental analyses and calculated parameters of ^{13}C NMR spectra for arable land
x-G average value of elemental analysis and calculated parameters of ^{13}C NMR for grassland



H/C –atomic ratio H/C; O/C – atomic ratio; HA – humic acids
Figure 8. H/C versus O/C diagram for HA

Cambisol and Haplic Stagnosol are typical of these soil types and are in accordance with the published literature (Barančíková 2014). As evident from Table 2, decreasing of carbon and hydrogen in HA molecule after land conversion was determined. On the other hand, increase of oxygen and nitrogen in HA molecule was observed (Table 2).

Atomic ratios calculated from HA elemental composition is presented in the form of Van Krevelen diagram (Figure 8). The ratios H/C and O/C were used for graphic-statistical analyses. This is useful for evaluating the chemical processes influencing the HA formation. Van Krevelen diagram (Figure 8) indicated oxidation trend in grassland HA.

The results of ^{13}C NMR spectroscopy are very useful for HA structure (Novák & Hrabal 2011; Enev *et al*. 2014). ^{13}C NMR spectroscopy can quantitatively detect different carbon types (e. g. carbonyl, carboxyl, aromatic, olephinic, anomer, aliphatic carbon) in HA molecule. The most important ^{13}C NMR parameters are percentage of aliphatic (C_{alif}) and aromatic (C_{ar}) carbon, and aromaticity degree (α). Calculation of aromaticity degree was done according to Hatcher (1981). The predominance of aliphatic structure and relatively low aromaticity degree (Table 2) was characteristic for HA isolated from HC and HS. After land conversion, increasing of C_{alif} and decreasing of Car_{ar} take place in permanent grasslands (Table 2). These findings are consistent with the results of Perez *et al.*(2004), who reported that such trend is characteristic of a higher incorporation of plant residues in SOM. Ono *et al.* (2009) also found out that after incorporation of fresh organic matter content of aliphatic carbon increased and the aromaticity degree decreased. Gonzales-Perez *et al.* (2007) reported that HA isolated from uncultivated land contained less percentage of aromatic carbon in comparison with arable land.

CONCLUSIONS

Our results confirm that land use change is a main factor influencing content and quality of soil organic carbon. Soil monitoring system is a valuable tool for the observation of these changes. On the basis of statistical analysis, it can be concluded that after land conversion grasslands dispose by higher content of soil organic carbon in comparison to arable land. Higher input of plant and root residues in grassland soils is stabilizing SOC stock in the top soil. Also higher content of total nitrogen in grassland opposite to cropland was found. After conversion of land use, the changes also occurred in quality of soil organic matter. The obtained results show more labile and less mature SOM of grassland to compare with arable soils. Higher input of plant residues and incorporation of fresh organic matter on grassland reflected in changes of humic acid chemical structure. The changes in HA chemical structure confirm the increase in aliphatic carbon content and decrease in aromaticity degree

Acknowledgments. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0243-11, No. APVV-14-0087, and No. APVV-0098-12.

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Received: December 15, 2015

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 62, 2016 (1): 19−27

Original paper

DOI: 10.1515/agri-2016-0003

OIL CONTENT AND FATTY ACIDS COMPOSITION OF POPPY SEEDS CULTIVATED IN TWO LOCALITIES OF SLOVAKIA

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LANČARIČOVÁ, A. – HAVRLENTOVÁ, M. – MUCHOVÁ, D. – BEDNÁROVÁ, A.: Oil content and fatty acids composition of poppy seeds cultivated in two localities of Slovakia. Agriculture (Poľnohospodárstvo), vol. *62*, 2016, no. 1, p. 19–27.

Oil content, fatty acids profile, acid and saponification values of poppy seeds grown on two localities of the Slovak Republic were evaluated in the study. Statistically significant effects of locality, genotype and their interaction ($P < 0.05$) for numerous descriptors were proved by non-parametric tests. Results confirmed that variation in the analysed parameters was influenced by the colour of seeds. Ochre variety Redy contained the highest oil level in both localities (49.9 and 52.4%) and linoleic acid level (74.3 and 71.6%). White-seeded Racek and Albín had the highest acid value (2.8 and 2.4% of free fatty acids) and grey-seeded Malsar and blue-seeded Maratón contained the highest saponification value. Buddha, a high-morphine poppy variety, differed significantly in all monitored parameters. High negative interrelation between linoleic and oleic acids levels was observed. Oil content was positively correlated with linoleic acid and negatively with oleic acid. Weather conditions at the end of vegetation influenced the accumulation of oil and essential linoleic acid.

Key words: poppy seed, fatty acid, oil, locality

Poppy (*Papaver somniferum* L.) is worldwide cultivated as a basic raw material for manufacture of pharmaceutically important narcotics as well as for production of seeds. In 2012, the Czech Republic was a leading producer of poppy seeds in the world with a total production of 12,814 tons (FAOSTAT 2014). On the other hand, the Slovak Republic reached a production of 296 tons. In Slovakia, poppy is cultivated mainly for oily seeds containing 50% of oil (Luthra & Singh 1989; Bozan & Temelli 2008). Seeds are used especially in the food industry as sprinklings and fillings in confectionary and bakery. Because of high content of polyunsaturated fatty acids (PUFAs), they are suitable in human nutrition. Dominant fatty acids in poppies are linoleic and oleic, next palmitic, stearic and *alpha*-linolenic acids (Nergiz & Otles 1994; Azcan *et al*. 2004). Currently, fatty acids attract a great attention thanks to their beneficial effects on human health. Due to high level of PUFAs, poppy seeds and its products are very susceptible to auto-oxidation, resulting in unpleasant odour and bitter taste. Therefore, detailed chemical analysis of oil quality is required for its industrial and nutritive use. Oils of high quality are

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suitable for food industry and cosmetics. Determination of current quality of oil with regard to lipid oxidation can be achieved using procedures as acid value, peroxide value, p-anisidine value. The acid value (free fatty acids) is an important parameter of quality of seed oil and reflects the amount of free fatty acids hydrolysed from triacylglycerols (Berezin *et al*. 1996). Free fatty acids are a key indicator of hydrolytic degradation associated with off flavour and oil changes (Atinafu & Bedemo 2011). Saponification value is the average molecular weight of fatty acids, bonded and unbonded acids, present in the oil. Oils with the higher saponification value are suitable for use in cosmetics (Tamzid *et al*. 2007).

The chemical composition of poppy seeds is influenced by many factors, for example, genotype, environment – locality and year of cultivation. Many studies (Vašák 2010; Azcan *et al*. 2004; Valizadeh *et al*. 2014) showed that colour of seed significantly affected some substances of poppy seed (protein, oil and fatty acids). Rahimi *et al*. (2011) observed high variability in oil and fatty acid amounts of 18 different Turkish poppy varieties. Similarly, Rahimi *et al*. (2015) evaluated the significant effect of locality on the oil content and fatty acids levels of four poppy genotypes cultivated in two localities of Turkey. According to Hlinková *et al*. (2012), quality of oil was influenced by environmental conditions of poppy cultivation.

The trend in poppy seed cultivation and exploitation is increasing. Therefore, the aim of the study was to evaluate the basic quality parameters of the seed such as oil content, fatty acids composition, chemical parameters [acid value (AV), free fatty acids (FFAs), respectively, and saponification value (SV)] in selected poppy varieties cultivated in two localities of Slovakia. The study presents chemometric evaluation of the influence of genotype and locality of cultivation on levels of analysed descriptors. Moreover, the interrelations among studied properties were examined. The colour of poppy seeds in relation to the descriptors was also investigated in this study. For breeders, those results served as valuable information concerning the appropriate conditions of poppy cultivation in Slovakia. Moreover, genotypes with the higher 'added value' could be suitable material for food industry.

MATERIAL AND METHODS

Field trials were conducted on two localities in Slovakia (Research and Breeding Station at Malý Šariš (east Slovakia) and Vígľaš-Pstruša (middle Slovakia) in 1 year (2011). Locality Malý Šariš is 21°10' East longitude, 49°00' Northern latitude, 310 m a.s.l., has an annual precipitation 624 mm, an average annual temperature of 8.1°C. Locality Vígľaš-Pstruša is 375 m a.s.l., 19º17'37'' East-West longitude, 48°33'19'' North-West width, has an annual precipitation 666 mm, an average annual temperature of 8.0°C. Malý Šariš has a rich content of humus (2.27%) and Stagni-Haplic Luvisol. The soil was characterised by high content of phosphorus (158 mg/kg), good content of potassium (276 mg/kg) and magnesium (241 mg/kg), with pH 6.1. The type of soil in Vígľaš-Pstruša is Stagni-Haplic Luvisol with the lower humus content (1.6%) than locality Malý Šariš. The soil was characterised by high content of phosphorus (138 mg/kg), fair content of potassium (146 mg/kg) and high content of magnesium (275 mg/kg), with pH 5.9.

Table 1

Selected characteristics of evaluated poppy genotypes (country of origin and year of registration)

Genotype	Country of origin	Year of registration
Bergam	SK	1998
Gerlach	SK	1990
Major	SK	2002
Malsar	SK	2002
Maratón	SK	2000
Opal	SK	1995
Orfeus	CZ	2009
Aristo	AT	2006
Buddha	HU	2004
Albín	SK	1991
Racek	CZ	2008
Redy	CZ	2008
MS ZB-3	SK	N
MS 387	SK	N
MS 423	SK	N

N = not registered genotype

Fifteen poppy genotypes (*Papaver somniferum* L.) were cultivated in two localities in randomised block design with four replications. Eight varieties (Bergam, Gerlach, Major, Malsar, Maratón, Opal, Albín, Orfeus) were listed at that time in the List of Registered Varieties of the Slovak Republic (ÚKSÚP 2011). Four varieties (Aristo, Buddha, Racek, Redy) are of European origin and three samples are breeding lines (MS ZB-3, MS 387, MS 423). Four colour types, ochre (Redy), white (Albín and Racek), grey (Malsar, restricted variety) and blue were used (Table 1). The seeds were sown on 26 March 2011 (at Vígľaš-Pstruša) and 29 March 2011 (at Malý Šariš). The date of harvest was 6 August 2011 at Malý Šariš and 16 August 2011 at Vígľaš-Pstruša. In both localities, plants were treated during the vegetation with herbicides and insecticides on the basis of the current spectrum of weeds and pests. Pre-emergence weed control was provided by systemic herbicide Callisto 480 SC (active substance, mesotrione). As postemergence control was used Laudis OD (active substance, tembotrione) in combination with Starane 250 EC (active substance, fluroxypyr). Against animal pests was applied insecticide Karate Zeon 5 CS (active substance, lambda-cyhalothrin) used as mix with plant stimulator Atonik (3 aromatic nitro compounds). Against gramineous weed species was used Garland Forte (active substance, propaquizafop).

The locality Malý Šariš in 2011 was characterised by the large misalignments of precipitation during the growing season (long-term drought during the germination and in early growth stages of the plant and heavy precipitation at the end of vegetation). Average temperatures for the months from April to July were 0.95ºC above the long-term average. At Vígľaš-Pstruša, precipitation and temperature in months April to July were higher than the long-term average. The end of vegetation was characterised by heavy precipitation. Comparison of temperature and precipitation of experimental sites are given in Table 2.

For oil content determination, a method by Soxhlet according to the norm (STN 461011-28) was used in two replications. To evaluate fatty acids composition by gas chromatography (GC), the method of fatty acids methyl esters preparation was used according to Christoperson and Glass (1969). Fatty acids were analysed as their methyl esters by GC (GC-6890 N, Agilent Technologies) using capillary column DB-23 and FID detector under a temperature gradient (Čertík & Ješko 2006). Fatty acids were identified by authentic standards of C4–C24 fatty acids methyl esters mixture (Supelco, USA) and by ChemStation 10.1 (Agilent Technologies). The degree of fatty acids unsaturation (UI) was calculated in D/mole: UI = [1 (% monoene) +2 (% diene) +3 (% triene)]/100 (Čertík & Šajbidor 1996).

AV (calculated as a percentage of FFAs in oil) was determined according to American Oil Chemists' Society (AOCS) Official method: AV (1998). SV of oil was determined using a method ASTM D464 (2010).

Chemometrical data analysis was carried out to discover statistically significant differences among poppy samples according to their genotype and locality of cultivation by means of general linear model (GLM) approach and alternatively by appropriate

Table 2

Meteorological conditions of experimental sites in 2011

Month	Temperature [°C]	Precipitation [mm]	Temperature [°C]	Precipitation [mm]
Locality	Malý Šariš		Vígľaš-Pstruša	
March	3.5	22.1	4.3	8.1
April	10.6	14.8	10.8	55.0
May	14.4	56.8	13.5	61.2
June	18.3	98.7	17.5	85.0
July	18.4	165.0	20.5	123.2
August	19.8	18.4	17.6	34.7

non-parametric tests. Significant correlations among studied descriptors were done using correlation analysis, specifically non-parametric Spearman correlation analysis. The principal component analysis (PCA) was applied to detect the natural grouping of samples and interrelations between analysed descriptors. Statistical data treatment was performed using SPSS v. 19.0 and STATISTICA v. 10.

RESULTS AND DISCUSSION

Malý Šariš was characterised by total higher oil values compared to Vígľaš-Pstruša (Figure 1). However, in both localities, the highest oil content had the ochre variety Redy (49.9% in Vígľaš-Pstruša and 52.4% in Malý Šariš). Moreover, white-seeded varieties were characterised by the higher oil content compared to blue and grey varieties. This result was confirmed in many studies (Azcan *et al.* 2004; Eklund & Agreen 1975). Different colours of poppy seed are related to the anatomical structure of the outer layers. White-seeded varieties are characterised by thin seed coat and a high oil content (Vašák 2010). Özcan & Atalay (2006) showed that oil content in poppy seeds depends on variety and ranged from 32.4 to 45.5%. We assume that oil content is strong genotypic trait. Varieties with white and ochre colour of seed contained the highest oil level, irrespective of locality.

The data in Table 3 show that white seeds contained the highest AV (2.4 and 2.8% of FFAs) at both localities. Our results are in agreement with the Özcan & Atalay (2006) and Wagner *et al.* (2003), which determined 1.6–3.2% of FFAs in selected varieties. The oxidative and chemical changes during storage of the oil are characterised by an increase in AV, amount of FFAs in the oil, respectively (Perkins 1992). Our results detected the relatively low hydrolytic and lipolytic activities in oil. The maximum limit of 2.0% of FFAs is reported by Codex Alimentarius (1993).

Varieties registered in the List of Registered Varieties in Slovakia (ÚKSÚP 2011) were characterised by higher SV of oil (Table 3) in range of 174.8 mg KOH/g of oil (Major, Malý Šariš) to 204.7 mg KOH/g of oil (Malsar, Vígľaš-Pstruša). No significant differences were found between localities. Our results were different compared to Azcan *et al.* (2004), which determined higher value, 234 mg KOH/g of oil. Our results indicated that selected poppy oils contained the higher levels of low molecular weight fatty acids. We assume that analysed oils have a suitable potential for industrial application, particularly in the manufacture of soaps and cosmetics.

The major fatty acids presented in poppy oil were linoleic (C 18:2), oleic (C 18:1) and palmitic (C 16:0) acids (Table 4). According to the literature, poppy oils contain 50–70% of linoleic acid,

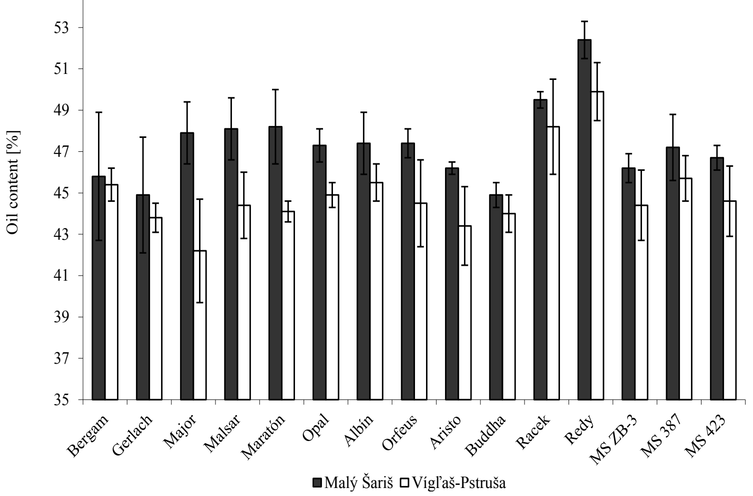

Figure 1. Average oil content [%] in poppy seeds grown in two localities in one year (Malý Šariš andVígľaš-Pstruša)

16–30% of oleic acid and 6–16% of palmitic acid (Nergiz & Otles 1994; Azcan *et al*. 2004). In general, levels of C 18:2 and C 16:0 were higher in Malý Šariš compared to Vígľaš-Pstruša. For C 18:1 was the situation opposite, higher amounts were detected in Vígľaš-Pstruša. Variety Buddha (high content of morphine) was characterised by the highest level of C 16:0 and C 18:1. The highest level of C 18:2 was detected in ochre and in white-seeded poppies. Equally to our results, white-seeded poppies contain higher levels of linoleic acid compared to blue ones (Nergiz & Otles 1994; Azcan *et al*. 2004). Absolutely dominant C 18:2 is responsible for the biosynthesis of arachidonic acid and some prostaglandins. Diet enriched with C 18:2 in patients with diabetes caused lower lipoprotein profile (Heine *et al*. 1989). Stearic (C 18:0) and *alpha*-linolenic (C 18:3) acids were presented as minor fatty acids (data not shown). Levels of C 18:0 varied from 2% (Albín) to 2.4% (Aristo, Racek and Gerlach) in both localities. The highest level of C 18:3 contained Redy in both localities (0.9%), however, other genotypes contained very similar levels of C 18:3 (0.7–0.8%). C 18:3 belongs to the *omega*-3 fatty acids with positive effect on atherosclerosis, ischemic heart disease, inflammatory diseases and probably also for conduct disorder (Connor 2000). On the other hand, C 18:3 inclines extensively to auto-oxidation; therefore, its high level is an ineligible factor in food industry (Bajpai *et al*. 1999). Considering this fact, poppy seed is a suitable material to be used in the food industry since it contains relatively low amounts of this fatty acid. Arachidic (C 20:0), gadoleic (C 20:1) and palmitoleic (C 16:1) acids were presented in amounts of 0.1–0.2%. From the levels of fatty acids was detected their high unsaturation in analysed oils. Average values of unsaturation index (UI) were 1.62 (Malý Šariš) and 1.59 (Vígľaš-

Table 3

Chemical properties of poppy seed oils extracted from seeds grown on two localities, Malý Šariš and Vígľaš-Pstruša

Descriptor	SFA [%]		MUFA [%]		PUFA [%]		FFA [%]		SV [mg KOH/g of oil]	
Locality	Malý Šariš	Vígľaš-Pstruša	Malý Šariš	Vígľaš-Pstruša	Malý Šariš	Vígľaš-Pstruša	Malý Šariš	Vígľaš-Pstruša	Malý Šariš	Vígľaš-Pstruša
Bergam	11.0^{+}	10.7^{+}	16.8^{+}	21.0^{+}	72.2^{+}	68.3^{+}	2.2	2.3	180.1	188.4
Gerlach	11.0	10.9	15.9^{+}	19.6^{+}	73.1^{+}	69.5^{+}	2.0	2.1	183.1	183.4
Major	11.2	11.1	17.4^{+}	19.9^{+}	71.4^{+}	69.0^{+}	2.0^{+}	2.3^{+}	174.8	187.5
Malsar	11.3	11.4	16.3^{+}	19.5^{+}	72.4^{+}	69.1^{+}	2.2^{+}	1.7^{+}	179.3	204.7
Maratón	11.1	11.0	16.2^{+}	19.3^{+}	72.7^{+}	69.7^{+}	1.8	1.4	199.4	196.8
Opal	10.6	10.6	16.3^{+}	19.3^{+}	73.1^{+}	70.1^{+}	1.7^{+}	1.3^{+}	190.6	204.3
Orfeus	10.8	10.8	16.3^{+}	19.1^{+}	72.9^{+}	70.0^{+}	1.9^{+}	1.1^{+}	174.8^{+}	198.4^{+}
Aristo	12.0^{+}	11.6^{+}	17.1^{+}	19.0^{+}	70.9^{+}	69.4^{+}	2.3^{+}	1.4^{+}	175.6^{+}	198.7^{+}
Buddha	12.6^{+}	12.3^{+}	19.7^{+}	23.7^{+}	67.7^{+}	64.0^{+}	2.2	1.8	173.5^{+}	198.8^{+}
Albín	11.2^{+}	11.0^{+}	14.2^{+}	16.8^{+}	74.6^{+}	72.2^{+}	2.4	2.2	185.7^{+}	193.4^{+}
Racek	11.6^{+}	11.4^{+}	13.6^{+}	16.6^{+}	74.8^{+}	72.0^{+}	2.1	2.8	185.2	190.0
Redy	10.9	10.9	13.9^{+}	16.6^{+}	75.2^{+}	72.6^{+}	2.1^{+}	2.6^{+}	181.0	182.2
MS ZB-3	11.5^{+}	11.1^{+}	15.9^{+}	18.9^{+}	72.6^{+}	70.1^{+}	2.0^{+}	2.4^{+}	182.8	179.1
MS 387	11.3	11.3	18.5^{+}	20.2^{+}	70.2^{+}	68.5^{+}	1.9	2.0	176.9	178.1
MS 423	11.2	11.1	16.6^{+}	19.3^{+}	72.3^{+}	69.6^{+}	2.1	2.0	187.0	184.9

$^{+}$statistically significant differences ($P < 0.05$) between Malý Šariš and Vígľaš-Pstruša for particular genotypes tested by Mann–Whitney test

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; FFA – free fatty acids; SV – saponification value of oil

Pstruša). The ratios of oleic acid/linoleic acid (data not shown) were calculated and included to the final statistical evaluation. Our results showed that high morphine variety had the highest amount of saturated fatty acids (SFAs), 12.5% in average and monounsaturated fatty acids (MUFAs), 21.7%. Redy disposed by the highest PUFAs in both localities. However, white-seeded varieties Racek and Albín contained a high value of PUFA (difference in tenth of a percent compared to Redy).

According to climatic conditions, there is an assumption that analysed quality parameters could be affected by temperature and precipitation. Malý Šariš was characterised by heavy precipitation at the end of vegetation (June and July) reported in Table 2. However, the average temperature at the end of vegetation was higher in Vígľaš-Pstruša (19.1°C). Since the oil content and the level of C 18:2 were higher in Malý Šariš, it could be assumed that lower temperatures and greater precipitation can cause increased accumulation of oil, however, more research needs to be done in this area. Similarly, according Canvin (1965), fatty acid composition was not affected by changes in temperature. However, the amount of higher unsaturated fatty acids decreased with increasing temperature. The fatty acids are important components providing structural barriers against changes in the environment (Beisson *et al*. 2007). Their levels are influenced by the environmental stress and salinity and heave metals in soil too (Maksymiec 2007; Mikami & Murata 2003).

The interaction effect genotype × locality was found to be statistically significant ($P < 0.05$) regarding all analysed descriptors by GLM. Consequently, locality was found statistically significant ($P < 0.05$) for numerous descriptors when considered each genotype category individually (Tables 3 and 4). Besides, the factor genotype was determined as statistically significant ($P < 0.05$) in all investigated descriptors for both levels of locality considered separately. Similarly, Sethi *et al*. (2006) confirmed the statistically significant effect of genotype and locality on the yield and oil content of poppy seeds.

Statistically significant correlations were found for numerous pairs of descriptors (Table 5). The highest negative correlations were observed between C

Table 4

Levels of dominant fatty acids (in %) in poppy seeds grown in two localities, Malý Šariš and Vígľaš-Pstruša

Descriptor	C 16:0		C 18:1		C 18:2	
Locality	Malý Šariš	Vígľaš-Pstruša	Malý Šariš	Vígľaš-Pstruša	Malý Šariš	Vígľaš-Pstruša
Bergam	8.7 ± 0.06^{+}	8.3 ± 0.17^{+}	16.6 ± 0.67^{+}	20.7 ± 0.43^{+}	71.4 ± 0.65^{+}	67.6 ± 0.33^{+}
Gerlach	8.5 ± 0.01^{+}	8.3 ± 0.06^{+}	15.6 ± 0.19^{+}	19.4 ± 0.17^{+}	72.4 ± 0.2^{+}	68.7 ± 0.11^{+}
Major	8.8 ± 0.03^{+}	8.7 ± 0.06^{+}	17.1 ± 0.39^{+}	19.6 ± 0.26^{+}	70.6 ± 0.42^{+}	68.2 ± 0.40^{+}
Malsar	9.0 ± 0.05	9.0 ± 0.06	16.1 ± 0.14^{+}	19.2 ± 0.35^{+}	71.6 ± 0.17^{+}	68.3 ± 0.27^{+}
Maratón	8.7 ± 0.02^{+}	8.5 ± 0.09^{+}	15.9 ± 0.53^{+}	19.1 ± 0.12^{+}	72.0 ± 0.58^{+}	68.9 ± 0.08^{+}
Opal	8.2 ± 0.10	8.1 ± 0.14	16.1 ± 0.46^{+}	19.0 ± 0.31^{+}	72.4 ± 0.48^{+}	69.3 ± 0.45^{+}
Orfeus	8.4 ± 0.07	8.4 ± 0.13	16.1 ± 0.48^{+}	18.9 ± 0.2^{+}	72.1 ± 0.50^{+}	69.3 ± 0.14^{+}
Aristo	9.4 ± 0.02^{+}	9.0 ± 0.08^{+}	16.9 ± 0.17^{+}	18.8 ± 0.33^{+}	70.2 ± 0.19^{+}	68.6 ± 0.35^{+}
Buddha	10.1 ± 0.13^{+}	9.9 ± 0.12^{+}	19.4 ± 0.3^{+}	23.4 ± 0.48^{+}	66.9 ± 0.38^{+}	63.1 ± 0.61^{+}
Albín	9.1 ± 0.03^{+}	8.8 ± 0.32^{+}	13.9 ± 0.16^{+}	16.5 ± 0.74^{+}	73.8 ± 0.14^{+}	71.4 ± 0.73^{+}
Racek	9.1 ± 0.23^{+}	8.9 ± 0.22^{+}	13.3 ± 1.82^{+}	16.3 ± 0.21^{+}	74.1 ± 1.61^{+}	71.3 ± 0.34^{+}
Redy	8.6 ± 0.06	8.5 ± 0.24	13.6 ± 0.16^{+}	16.3 ± 0.38^{+}	74.3 ± 0.17^{+}	71.6 ± 0.46^{+}
MS ZB-3	9.1 ± 0.12^{+}	8.7 ± 0.11^{+}	15.6 ± 1.60^{+}	18.6 ± 0.51^{+}	71.8 ± 1.47^{+}	69.2 ± 0.53^{+}
MS 387	9.0 ± 0.04	8.9 ± 0.22	18.2 ± 0.46	19.9 ± 1.21	69.5 ± 0.39^{+}	67.7 ± 0.95^{+}
MS 423	8.8 ± 0.04^{+}	8.7 ± 0.04^{+}	16.3 ± 0.37^{+}	19.0 ± 0.29^{+}	71.5 ± 0.44^{+}	68.8 ± 0.26^{+}

$^{+}$statistically significant differences ($P < 0.05$) between Malý Šariš and Vígľaš-Pstruša for particular genotypes tested by Mann–Whitney test

C 16:0 – palmitic acid; C 18:1 – oleic acid; C 18:2 – linoleic acid

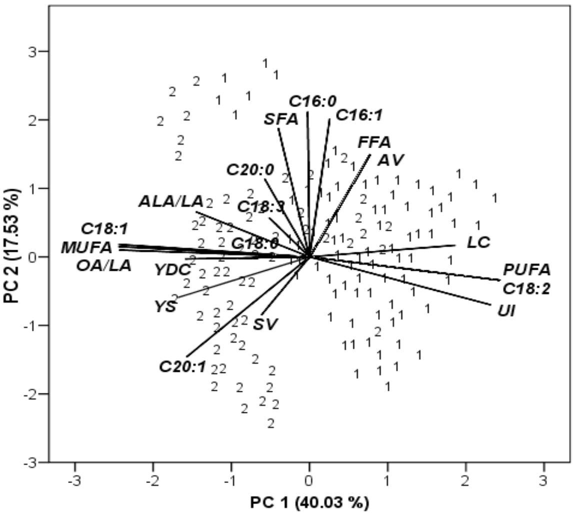

Figure 2. PCA bi-plot showing the mutual position of original variables along with the groups of poppy samples labelled according to locality of cultivation (1 – Malý Šariš, 2 – Vígľaš-Pstruša). PCA: principal component analysis.
OA/LA – oleic/linoleic acid ratio; MUFA – monounsaturated fatty acids; C 18:1 – oleic acid; ALA/LA – *alpha*-linolenic/linoleic acid ratio; YDC – yield of dry capsules; YS – yield of seed; C 20:1 – gadoleic acid; C 18:0 – stearic acid; SV – saponification value; C 20:0 – arachidic acid; C 18:3 – *alpha*-linolenic acid; SFA – saturated fatty acids; C 16:0 – palmitic acid; C 16:1 – palmitoleic acid; FFA – free fatty acids; AV – acid value; LC – oil content; C 18:2 – linoleic acid; PUFA – polyunsaturated fatty acids; UI – unsaturation index

18:1 and C 18:2 and consequently, between MUFAs and PUFAs. This correlation indicates a competitive relationship between oleic and linoleic acids in the biosynthesis of fatty acids in the poppy seeds. The biosynthesis of fatty acids is a very complex process; all plants produce fatty acids in pathway with precursor acetyl-CoA. PUFAs are synthesised in high plants by both, eukaryotic and prokaryotic pathways (Browse *et al.* 1986). According to fatty acids profile in our analysed poppies, C 16:0 is elongated to C 18:0 and then stearoyl-CoA-desaturase catalyses conversion of C 18:0 to C 18:1. Desaturases (Δ^{12} and Δ^{15}) catalyse a consecutive conversion from oleic acid to linoleic acid and from linoleic to *alpha*-linolenic acid. C 20:0 and also C 20:1 are formed by the elongation of stearic and oleic acids. Bajpai *et al.* (1999) confirmed significant positive correlation between yield and oil content and Luthra and Singh (1989) found positive correlations between C 18:0 and C 18:1 and between C 18:2 and C 18:3. Small discordances with our results are presumably occasioned by different poppy material selection and as a consequence, the differences in biosynthesis pattern of fatty acids may occur with respect to the particular genotype and environment.

PCA showed that natural grouping of samples in relation to their locality of cultivation was obvious (Figure 2). Consequently, we concluded, that the quality of poppy seeds depends on their genotype and especially on the locality of growing, that is,

Table 5

Reduced correlation (Spearman correlation coefficients ($P < 0.05$)) table for all poppy samples ($n = 180$)

Descriptor	LC	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3	C 20:1	SFA	MUFA	PUFA	YDC
C 18:0	–0.26	0.08									
C 18:1	-0.67^{+}	–0.06	0.29								
C 18:2	0.67^{+}	–0.10	–0.34	-0.97^{+}							
C 18:3	–0.30	–0.08	–0.15	0.24	–0.23						
C 20:1	-0.51^{+}	-0.50^{+}	0.30	0.59^{+}	-0.52^{+}	0.14					
SFA	–0.05	0.94^{+}	0.36	0.04	–0.21	–0.11	–0.38				
MUFA	-0.67^{+}	–0.06	0.29	1.00^{+}	-0.98^{+}	0.24	0.59^{+}	0.04			
PUFA	0.66^{+}	–0.11	–0.35	-0.97^{+}	1.00^{+}	–0.21	-0.52^{+}	–0.22	-0.97^{+}		
YDC	–0.44	0.00	0.20	0.50^{+}	-0.52^{+}	0.48	0.49	0.08	0.50^{+}	-0.51^{+}	
YS	–0.46	–0.26	0.21	0.56^{+}	-0.54^{+}	0.39	0.69^{+}	–0.16	0.56^{+}	-0.53^{+}	0.76^{+}

$^{+}$highly significant correlation ($P < 10^{-6}$)
MUFA – monounsaturated fatty acids; C 18:1 – oleic acid; YDC – yield of dry capsules; YS – yield of seed; C 20:1 – gadoleic acid; C 18:0 – stearic acid; C 18:3 – *alpha*-linolenic; SFA – saturated fatty acids; C 16:0 – palmitic acid; LC – oil content; C 18:2 – linoleic acid; PUFA – polyunsaturated fatty acids

climatic conditions. Another separated cluster of samples represents high morphine variety Buddha, characterised by lower C 18:3 and oil content (LC) and higher C 16:0 contents compared to all other genotypes. Concerning this cluster individually, poppy samples from Malý Šariš are also separated from Vígľaš-Pstruša. Hence, the PC 1 represents original variables OA/LA, MUFA, C 18:1, PUFA, and C 18:2, UI as well as oil content (LC), yield of seed and capsules (YDC and YS) with the highest loadings for this vector. In addition, the PC 2 represents the original descriptors C 16:0, C 16:1, SFA and C 20:1 and contributes to the separation of samples in relation to their genotype. Also, samples denoted with number 2, which are mixed in group of locality 1 (Malý Šariš) belongs to special genotypes – the white- and ochre-seeded varieties characterised with higher levels of C 18:2 and LC, causing the aggravated stability in storage. On the other hand, white- and ochre-seeded varieties differed in terms of their lower levels of C 18:1, YDC and YS.

According to the result, it can be concluded the positive correlation of LC with C 18:2 (and therefore, also with PUFA and UI) and their negative correlation with C 18:1, calculated variables MUFA, OA/LA and interestingly also, with YDC and YS. In other words, by increasing YDC and YS, the content of oil and linoleic acid level in seeds is decreasing, as it was shown also in correlation analysis.

CONCLUSIONS

Genotypes cultivated at locality Malý Šariš were characterised by higher oil content, concentrations of palmitic and linoleic acid. On the other hand, the situation was opposite at Vígľaš-Pstruša. Genotypes cultivated at Vígľaš-Pstruša contained the lower oil content, lower concentrations of palmitic and linoleic acid, but the higher amount of oleic acid. Genotypes with ochre and white colour of seed contained the highest oil content in both localities. However, white colour of seed was associated with higher AVs of oil, reflecting low anti-oxidative stability. Our results indicated that genotypes listed in the List of Registered Varieties of Slovak Republic were characterised by higher SV of oil. As major fatty acids were detected linoleic, palmitic and oleic acids. High-morphine genotype Buddha contained the highest levels of palmitic and oleic acid. Factors genotype, locality and their interactions were statistically significant ($P < 0.05$). High negative interrelation between linoleic and oleic acids levels was determined.

Acknowledgements**:** The work was supported by the projects APVV-0248-10 and VEGA 1/0233/12 from the Ministry of Education of the Slovak Republic.

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Received: March 10, 2015

Agriculture (Poľnohospodárstvo), 62, 2016 (1): 28−40
DOI: 10.1515/agri-2016-0004
Original paper

ANALYSIS OF RELATIONS BETWEEN CROP TEMPERATURE INDICES AND YIELD OF DIFFERENT SUNFLOWER HYBRIDS FOLIAR TREATED BY BIOPREPARATIONS

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KOVÁR, M. – ČERNÝ, I. – ERNST, D: Analysis of relations between crop temperature indices and yield of different sunflower hybrids foliar treated by biopreparations. Agriculture (Poľnohospodárstvo), vol. *62*, 2016, no. 1, pp. 28–40.

The application of biological active preparations (BAPs) and remote-sensing control in the management of agronomic intervention are an important part of successful crop cultivation. The effects of foliar application of two BAPs (containing amino acids or Abiestins®) on yield and yield-forming, as well eco-physiological traits calculated from infrared thermographs data (crop water stress index, CWSI and index of stomatal conductance, Ig) of three hybrids of sunflower were studied in field poly-factorial experiments, realised during two years (2012 and 2013). The results showed that the application of selected BAPs has contributed to an increase of the sunflower yield, in particular through an increase in the weight of thousand seeds ($rp = 0.761$, $P < 0.001$). Similarly, oil content in achenes was significantly higher in treatments with BAPs, mainly with preparation containing free amino acids. The study describes the quantitative relationship between yield and quality of sunflower production ($rp = –0.41$, $P < 0.01$). Selected hybrids of sunflower in two growth stages showed the significant differences in CWSI and Ig (both at $P < 0.01$), respectively. An analysis of negative linear relation between the yield of achenes and CWSI ($rp = –0.654$, $P < 0.001$) confirmed that higher value of plant stress resulted in a smaller yield and vice-versa. The opposite trend was observed between yield and Ig index ($rp = 0.576$, $P < 0.001$). The data obtained from IR thermography can be used for monitoring the physiological health of sunflower plants, as well in potential prediction and control of yield.

Key words: infrared thermography, CWSI, yield, oil content, foliar preparations, sunflower

Sunflower currently is the world’s fourth most important oil crop with a harvested area of about 25 million hectares in which 36 million tons of achenes are produced on average. France is the largest producer in EU countries, with a production of around 1.6 million tons. The production in Slovakia is around 0.2 million tons. The average world production of sunflower is 1.42 t/ha approximately. The crop area, yield and overall production of sunflower have been relatively stable in Slovakia in the past five years. The sunflower-cultivated area was 0.084 million hectares, the yield reached 2.3 t/ha and the total production reached 0.196 million tons in Slovakia in 2013 (FAO 2013). Complete and homogeneous stands provide high yield while respecting limiting factors of productivity (Pasda & Diepenbrock 1991; Zheljazkov *et al*. 2008). The limiting factors of productivity of sunflower mainly are soil and habitat conditions – geographic location, altitude, soil quality and its properties (Helmy & Ramadan 2009), climate and weather conditions − temperature, precipitation, year (Wanjari *et al.* 2001), the ability of plants − photosynthetic activity, respiration, transpiration, size of assimilation system, the genetic basis of the hybrid, resistance to adverse factor and creation and reduction of yield

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forming elements (Gibbs 2004; Dalai *et al*. 2008) and agricultural engineering and farming methods − crop rotation, forecrop, soil cultivation, seed treatment, sowing rate, nutrition and fertilisation, application of biologically active substances, sowing, protection against diseases and pests and the quality of the harvest (Marschner 2003; Cerkal *et al*. 2011; Elezovic *et al*. 2012).

The agricultural practice that is successfully employed to eliminate the negative effects of stressful situation on crop productivity is the application of biologically active preparations (BAPs) (Calvo *et al*. 2014). Is well documented the positive effect of foliar application of BAPs with amino acids as an active substance on yield of many crops (Tejada & Gonzales 2003; Jablonskyte-Raščе *et al*. 2013), including sunflower (Rauf 2008; Mátyás *et al*. 2014). It has been observed that application of BAPs stimulate photosynthetic performance and anti-oxidative defence metabolism, in addition to water, light and mineral use efficiency, as well uptake of mineral nutrition. Finally, these plant responses minimise the negative effects of environmental stresses on crop productivity (Rhodes *et al*. 1999; Oosterhuis & Robertson 2000; Djanaguiraman *et al*. 2004; Kovár & Černý 2012).

One approach that finds application in the management of the irrigation system, as well the screening of biological material and optimisation of agronomic intervention, is the measure of the temperature of leaf / crop (Jones *et al*. 2009). These approaches use thermometers in contact with the leaves, or (especially today) spot imaging and infrared cameras. The temperature of the sheet resulting from the plant and the power balance of the soil and the atmospheric conditions change transpiration, which is controlled by diffusion of water vapour through the vents, and thus stomatal conductivity (g_S). The basic idea is that closure of stomata (decrease in g_S), which occurs under the conditions of stress, resulting in the reduction of heat energy dissipation and the temperature rise in the leaf lamina. Infrared (IR) thermography of plant leaf / crop is a good tool for assessment the stomatal conductivity, and the detection of water stress plants, efficient use of water management and irrigation (Jones 1999; Jones *et al*. 2009). Calculation of stress indices, which are based on measurements of leaf temperature / vegetation, is widespread in practice. The most common indices used in field conditions are: stress degree days index (SDD), temperature differential (ΔT, the temperature difference between leaf and air), the crop water stress index (CWSI), water deficit index (WDI) and the stomatal conductivity index (Ig) (Reginato 1983; Jones *et al*. 2009; Padhi *et al*. 2012). Several studies from past decades have been used the CWSI index to describe the water status of the crop grown at field (Idso *et al*. 1981; Jackson *et al*. 1981; O´Shaughnessy *et al*. 2011; Argyrokastritis *et al*. 2015). Taghvaeian *et al*. (2014) shows that CWSI index can be used for effective monitoring of water stress and scheduling irrigation in sunflower. This study concluded that CWSI strongly correlated with canopy growth intensity. The increase of CWSI value under crop water stress results in yield reduction. Previously, Gardner *et al.* (1981) and Irmak *et al.* (2000) correlated grain yields of corn with differences in canopy temperature and CWSI, respectively. The polynomial relationship between CWSI and both yield and protein content in soybean seeds was reported (Candogan *et al*. 2013).

Currently, no study is available on the effect of exogenous application of BAPs on stomatal activity calculated from IR thermography of sunflower plants and their relationship to yield of the seed. Therefore, the objectives of this study were to evaluate the effect of foliar application of selected biologically active preparations on yield, yield-forming parameters and oil content in seeds of sunflower, as well to determine the relationship between yield and CWSI and Ig indices calculated from IR thermography in two different growth stages of sunflower plants.

MATERIAL AND METHODS

Plant material and treatments

This experiment was conducted in order to investigate the effects of year-round weather conditions, biological material (genotype) and treatment by foliar preparations (containing the free amino acids or Abiestins®) on selected yield-forming elements, yield and oil content in sunflower. The experiment was performed during 2012 and 2013 at the research fields of the Plant Biology and Ecology Centre, the Faculty of Agrobiology and Food Resources of the Slovak University of Agriculture (SUA) in Nitra, Slovakia (48°19'25.41'' N and 18°09'2.87'' E, altitude 250 m above sea level). The experimental area is situated in the maize-growing region (climatic region: warm; climatic sub-region: mild dry or dry; climatic zone: warm and dry, with mild winter and long sunshine) and soil is, according to FAO classification, silt loam Haplic Luvisol (WRB 2006; Šimanský & Kováčik 2015). The experiments were established by block method with a completely randomised design of experimental field plot trial (60 m^2 per one plot) in three repetitions. Forecrop of sunflower (*Helianthus annuus* L.) in seven-plot crop rotation was winter wheat (*Triticum aestivum* L.). Soil cultivation (stubble ploughing, autumn deep ploughing) and method of crop stand establishment (alternate row distance 0.70 m, distance in row 0.22 m) were performed in accordance with the principles of conventional technology of sunflower cultivation. The soil fertilisation was derived from soil agrochemical analysis for an expected yield of 3.0 t/ha. The plants were fertilised (i) in 2012 with nitrogen at the rate of 107 kg/ha urea, followed by 50 kg/ha single superphosphate and of 200 kg/ha KCl and (ii) in 2013 at the rate of 200 kg/ha DASA® 26/13, using fertiliser applicator FERTI (FPM Agromehanica, Boljevac, Serbia). Fertilisers containing P and K were applied to the soil during autumn with deep ploughing. Fertilisers containing N were applied to the soil during pre-sow ploughing in the spring. Climatic characteristics of the experimental area were obtained from the Meteorological Station of Horticulture and Landscape Engineering Faculty of Slovak University of Agriculture in Nitra (Figure 1).

Three hybrids of sunflower, NK Brio, NK Neoma and NK Ferti, were used in the experiment. Three variants were established as (i) control; (ii) variant with application of preparation containing the amino acid [9.3% of L-α amino acids (Asp, Ser, Glu, Gly, His, Arg, Thr, Ala, Pro, Cis, Tyr, Val, Met, Lys, Ile, Leu, Phe, Trp) and 2.1% of N, as well as 0.07% of zinc, 0.04% of manganase and 0.02% of boron] with trade name Terra-Sorb® Foliar (Biobérica S.A., Barcelona, Spain) and (iii) application of preparation containing the biologically active substances (Abiestins®, minimal content 40 g/L) from near polar plants with trade name Unicum® (Ekoland Europe s.r.o., Praha, Czech Republic). The control variant was without foliar preparations. Preparations were applied manually with pressurised hand sprayer (capacity 10 L) Gamma10 (Mythos Di Martino, Mussolente, Italy) twice during the growing season at the following rates: (a) application of Terra-Sorb® Foliar was done in two stages (first at 2−4 leaf stage and second 20 days after the first application), both in dose of 1.5 L/ha; b) application of Unicum® was done in two stages (first at 2−4 leaf stage and second during flowering), both in the dose of 200 mL/ha.

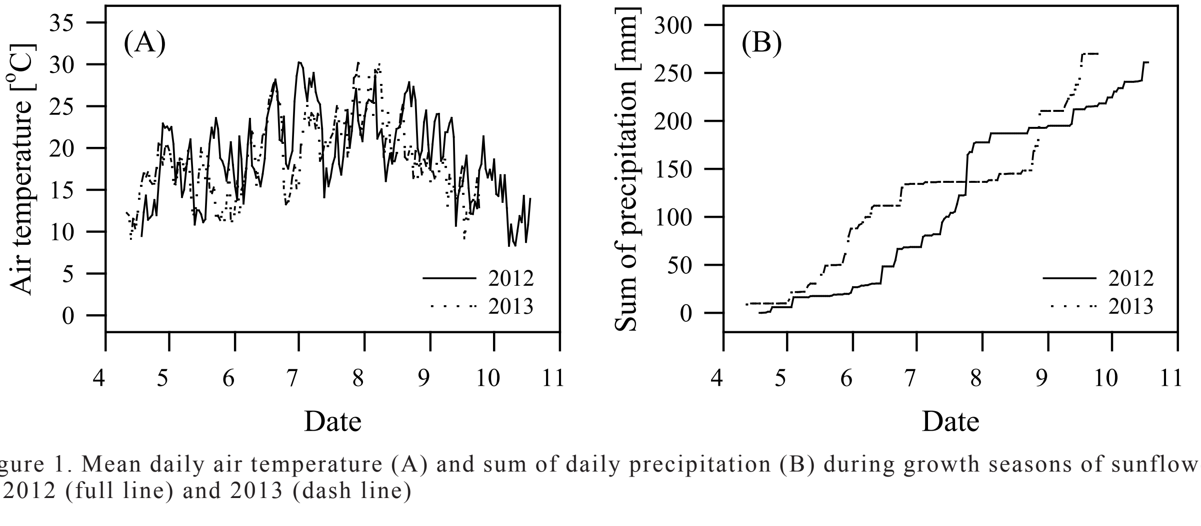

Figure 1. Mean daily air temperature (A) and sum of daily precipitation (B) during growth seasons of sunflower in 2012 (full line) and 2013 (dash line)

Calculation of yield-forming parameters and oil content measurement

Heads were harvested manually and taken to the laboratory, where yield-forming elements were determined. The harvest was performed by small-plot combine CLAAS (CLAAS GmbH & Co. KGaA, Harsewinkel, Germany). The yield of achenes harvested from experimental area was re-calculated to unit tonnes per hectare [t/ha]. Oil content in seed was determined by a standard method using the Soxhlet apparatus (Shahidi 2005). Prior to extraction, the seeds were mechanically crushed using a laboratory homogeniser to an average particle size of 1 mm. The direct oil extraction was performed using petroleum ether reagent at 60°C. Total extraction time during the analysis was 60 minutes (15 min direct extraction samples immersed in the extraction reagent and 45 min exposure to reagent vapours). After extraction, the crude oil was directly weighed and oil content was recalculated in the sample.

Calculation of CWSI and Ig indices

Measurement of leaf temperature (T^{leaf}) was carried out in two different stages under plant development, flowering and ripening (BBCH 63 and 87) by hand IR thermal camera EasIR-4 (Bibus AG, Fehraltorf, Switzerland). Thermal images were taken from mature leaves of 10 plants which underwent experimental treatment, between 11:00 and 13:00 hours SCER from a distance 2.0 m perpendicular to the lamina surface and at an elevation of 1.5 m. IR thermal camera uses uncooled FBA detector with 160 × 120 pixel elements, sensitive in the spectral range of 8–14 µm and angular field of view of 20.6 × 15.5°, with an accuracy ±2.0°C and thermal resolution ≤0.1°C. Emissivity was set at 0.95. Two references were used for measured minimal and maximal surface temperature. Crop imaging was taken from the continuous thermal images from two references surfaces. The wet and dry artificial surface of green colour was used to measure the minimal (T^{wet}) and maximal (T^{dry}) temperature. Thermal images were analysed with Guide IR Analyser (Bibus AG, Fehraltorf, Switzerland). Two lines were crossed over lamina and average leaf temperature (T^{leaf}) was determined. Crop water stress index (CWSI) and stomatal conductance index (Ig) were calculated from the following equations:

$$\text{CWSI} = \frac{(\text{T}^{\text{leaf}} - \text{T}^{\text{wet}})}{(\text{T}^{\text{dry}} - \text{T}^{\text{wet}})}$$

$$\text{Ig} = \frac{(\text{T}^{\text{dry}} - \text{T}^{\text{leaf}})}{(\text{T}^{\text{leaf}} - \text{T}^{\text{wet}})}$$

Statistical analysis

The experimental data were graphically assessed as mean values for each experimental member with the corresponding standard deviation. Statistical analysis was performed using Statistica software, version 10 (StatSoft, Inc., Tulsa, Oklahoma, USA). The normal distribution and homogeneity of experimental results was tested by Kolmogorov–Smirnov and Lavene's test. Statistical differences between the individual experimental members (genotype, treatment, and year) were analysed by multi-factorial ANOVA analysis and homogenous groups were identified using Duncan's post-hoc test at a significance level of $P < 0.05$. The correlation analysis between experimental traits was expressed by the Pearson correlation coefficient (r_p).

RESULTS AND DISCUSSION

Yield-forming parameters

Yield forming elements (head diameter, weight of head and weight of thousand achenes) were influenced by genotypes, treatments, year-round weather conditions and by its combined effect (Table 1, 2). The results of combined ANOVA showed high significant influence of year-round weather conditions on head diameter, weight of head and weight of thousand achenes (Table 2). This phenomenon has been reported by other researches (Amjed *et al*. 2011; Rauf *et al*. 2012; Mátyás *et al.* 2014). The results confirmed course of year-round weather conditions. Experimental years were unbalanced and very different. In view of the observed average monthly temperatures, as compared to the long-term climate normal, both years can be considered as above average. In terms of precipitation during the growing season for both experimental years, it was typical of an unequal course (Figure 1). Amjed *et al*. (2011) and Mátyás *et al.* (2014) observed that head diameter was high influenced by genotypes. Černý *et al*. (2013) found significant influence of genotype on weight of head. Rondanini *et al*. (2003)

Table 1

Production and physiological traits of three sunflower genotypes treated with two bio-preparations during the seasons of 2012 and 2013. Data represents the mean ± standard deviation.

Genotype	Treatment	Year	Head diameter [mm]	Weight of head [g]	TWA	Yield [t/ha]	Oil content [%]
NK Brio	Untreated	2012	186.7±20.8^{aA}	260.5±38.5^{bB+}	59.16±0.21^{cB+}	2.17±0.08^{aA+}	51.20±1.91^{aA+}
		2013	200.3±2.5^{cA}	170.1±2.0^{cC+}	34.60±0.83^{abB+}	1.84±0.05^{aC+}	61.84±0.50^{bC+}
	Terra-Sorb	2012	186.7±15.3^{aA}	242.5±24.5^{bAB+}	63.20±0.14^{cA+}	3.35±0.18^{aB+}	50.19±0.43^{aB+}
		2013	186.0±2.0^{cA}	118.4±2.5^{cA+}	29.23±2.00^{abA+}	2.08±0.10^{aB+}	58.47±0.50^{bA+}
	Unicum	2012	180.0±20.0^{aA+}	180.4±34.8^{bA+}	64.68±0.44^{cC+}	2.70±0.44^{aB+}	54.36±0.55^{aB+}
		2013	230.3±1.5^{cB+}	133.1±3.0^{cB+}	25.36±2.00^{abA+}	1.06±0.04^{aA+}	58.67±0.50^{bB+}
NK Neoma	Untreated	2012	173.3±15.3^{aA+}	207.1±14.6^{aB+}	54.03±0.11^{aB+}	2.62±0.07^{aA+}	52.31±1.71^{bA+}
		2013	192.0±2.0^{cA+}	127.3±2.2^{bC+}	39.05±4.92^{bB+}	2.78±0.02^{bC+}	52.25±0.60^{aC+}
	Terra-Sorb	2012	200.0±20.0^{aA}	164.6±31.1^{aAB+}	48.80±0.19^{aA+}	2.35±0.11^{aB+}	55.79±0.47^{bB+}
		2013	195.3±4.0^{cA}	116.2±2.0^{bA+}	26.93±0.90^{bA+}	1.49±0.04^{bB+}	52.75±0.53^{aA+}
	Unicum	2012	180.0±20.0^{aA}	174.9±19.4^{aA+}	64.32±0.17^{aC+}	3.13±0.37^{aB+}	56.70±0.46^{bB+}
		2013	190.0±2.0^{cB}	131.6±1.4^{bB+}	28.63±0.36^{bA+}	1.54±0.03^{bA+}	56.45±0.55^{aB+}
NK Ferti	Untreated	2012	166.7±5.77^{aA}	170.3±17.5^{aB+}	58.23±0.13^{bB+}	2.49±0.08^{aA+}	49.76±1.53^{aA+}
		2013	184.0±2.0^{aA}	108.5±1.9^{aC+}	26.44±0.33^{aB+}	2.29±0.02^{cC+}	55.72±0.47^{aC+}
	Terra-Sorb	2012	175.0±5.0^{aA+}	210.3±10.0^{aAB+}	56.16±0.17^{bA+}	2.91±0.22^{aB+}	54.62±0.40^{aB+}
		2013	190.0±2.0^{aA+}	119.2±1.9^{aA+}	28.41±0.41^{aA+}	2.11±0.02^{cB+}	54.06±0.38^{aA+}
	Unicum	2012	176.7±17.6^{aA+}	189.9±13.7^{aA+}	55.60±0.15^{bC+}	2.45±0.20^{aB+}	50.18±0.47^{aB+}
		2013	194.0±2.0^{aB+}	130.4±2.2^{aB+}	31.62±0.38^{aA+}	1.95±0.02^{cA+}	52.76±0.38^{aB+}

Table 1 continued

Genotype	Treatment	Year	CWSI-4	CWSI-6	Ig-4	Ig-6
NK Brio	Untreated	2012	0.50±0.02^{aB}	0.40±0.01^{aA+}	1.00±0.08^{aA}	0.72±0.00^{bC+}
		2013	0.47±0.02^{aB}	0.86±0.12^{bA+}	1.09±0.06^{bA}	0.39±0.05^{aA+}
	Terra-Sorb	2012	0.42±0.03^{aA+}	0.31±0.05^{aA+}	1.23±0.05^{aA}	0.74±0.02^{bA+}
		2013	0.39±0.00^{aA+}	0.79±0.23^{bAB+}	1.21±0.01^{bA}	0.38±0.05^{aB+}
	Unicum	2012	0.56±0.06^{aA+}	0.72±0.03^{aA+}	0.90±0.16^{aB+}	0.58±0.01^{bB+}
		2013	0.50±0.01^{aA+}	0.99±0.07^{bB+}	0.73±0.04^{bA+}	0.42±0.03^{aA+}
NK Neoma	Untreated	2012	0.53±0.03^{aB}	0.79±0.03^{cA+}	0.88±0.09^{aA}	0.56±0.01^{aC+}
		2013	0.54±0.05^{bB}	0.73±0.04^{aA+}	0.93±0.12^{aA}	0.42±0.03^{bA+}
	Terra-Sorb	2012	0.61±0.04^{aA}	0.88±0.03^{cA}	0.79±0.07^{aA+}	0.47±0.00^{aA+}
		2013	0.60±0.01^{bA}	0.81±0.13^{aAB}	0.66±0.02^{aA+}	0.36±0.03^{bB+}
	Unicum	2012	0.34±0.00^{aA+}	0.55±0.04^{cA+}	1.42±0.08^{aB+}	0.60±0.02^{aB+}
		2013	0.49±0.01^{bA+}	0.91±0.10^{aB+}	1.01±0.05^{aA+}	0.48±0.02^{bA+}
NK Ferti	Untreated	2012	0.52±0.00^{bB+}	0.49±0.03^{abA+}	0.92±0.01^{aA+}	0.67±0.02^{aC+}
		2013	0.59±0.02^{bB+}	0.82±0.13^{abA+}	0.78±0.06^{aA+}	0.37±0.05^{bA+}
	Terra-Sorb	2012	0.31±0.04^{bA+}	0.71±0.19^{bA}	0.96±0.11^{aA}	0.44±0.04^{aA+}
		2013	0.53±0.02^{bA+}	0.74±0.10^{abAB}	0.89±0.07^{aA}	0.38±0.02^{bB+}
	Unicum	2012	0.42±0.01^{bA+}	0.60±0.05^{bA+}	1.26±0.11^{aB}	0.57±0.04^{aB+}
		2013	0.47±0.03^{bA+}	0.77±0.07^{abB+}	1.10±0.12^{aA}	0.50±0.02^{bA+}

Note: Small and large letters indicate significant differences (Duncan´s test, $\alpha = 0.05$) between genotypes and treatments, respectively. $^{+}$Indicates significant differences between seasons.
TWA – weight of thousand achenes; CWSI – crop water stress index; Ig – stomatal conductance index; numbers -4 and -6 are measurements during vegetation period

observed that average weight of achenes is the decisive yield-forming factor that plays an important role in assessing the production potential of the sunflower hybrids. In this study, the significant influence of treatment on head diameter was not reported (Table 2), which probably results from water deficit in soil, mainly in 2013. It is well documented that drought-induced inhibition of growth is general response of plant to water deficit (Baldini *et al*. 1997; Rauf 2008; Jones *et al*. 2009). On the other hand, Kheybari *et al.* (2013) found the significant effect of foliar-applied amino acids on head diameter. We found significant influence of the treatment on weight of head and weight of thousand achenes (Table 2). The results of this study about the effect of applying different preparations on sunflower agree well with Hussain *et al*. (2012) and Mátyás *et al.* (2014). The combined effect of genotype × treatments had significant influence on head diameter. The weight of head and weight of thousand achenes were influenced highly significantly (Table 2). Mátyás *et al.* (2014) described non-significant influence of this interaction on head diameter and weight of head but high significant effect on weight of thousand achenes. The combined effect of genotype × year had not significant influence on head diameter. This result confirmed state of Mátyás *et al.* (2014). In weight of head and weight of thousand achenes, we found high a significant influence of this interaction (Table 2). Chimenti *et al.* (2001) reported that the production process of sunflower is a result of the properties of the parental lines of hybrids, course of weather conditions and its interaction, which agree with our findings. The combined effects of treatments × year had significant influence on head diameter and weight of head. The weight of thousand achenes was influenced highly significantly (Table 2). This observation agrees with the results of Mátyás *et al.* (2014). The interaction of genotypes × treatments × year did not have a significant effect on the head diameter and weight of head but it had a high significant influence on the weight of thousand achenes.

Table 2

Analysis of variance in production and physiological traits of sunflower genotypes treated with two biopreparations during the seasons of 2012 and 2013

		Head diameter [mm]	Weight of head [g]	TWA	Yield [t/ha]	Oil content [%]	CWSI-4	CWSI-6	Ig-4	Ig-6
Genotypes (G)	F	5.970	17.708	27.220	4.810	56.400	17.970	1.733	4.004	12.940
	P	0.006	0.000	0.000	0.014	0.000	0.000	0.191	0.027	0.000
Treatments (T)	F	2.140	4.637	28.560	12.17	6.600	25.410	6.119	13.849	51.240
	P	0.133	0.016	0.000	0.000	0.004	0.000	0.005	0.000	0.000
Year (Y)	F	23.040	228.110	5584.23	301.570	187.600	30.370	47.972	22.234	349.430
	P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G × T	F	2.880	9.809	28.820	35.820	32.700	60.540	8.290	50.873	26.600
	P	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G × Y	F	1.400	3.355	41.85	13.890	129.000	37.430	19.291	2.729	71.990
	P	0.240	0.046	0.000	0.000	0.000	0.000	0.000	0.078	0.000
T × Y	F	3.930	5.640	49.910	56.600	29.300	3.350	1.854	10.433	5.280
	P	0.029	0.007	0.000	0.000	0.000	0.046	0.171	0.000	0.010
G × T × Y	F	2.500	2.553	46.100	7.980	11.100	14.650	10.026	2.664	34.110
	P	0.060	0.056	0.000	0.000	0.000	0.000	0.000	0.048	0.000

Note: greyscale of cells indicates significant differences at level < 0.05 (light-grey), < 0.01 (medium-grey) and < 0.001 (dark-grey). TWA – weight of thousand achenes; CWSI – crop water stress index; Ig – stomatal conductance index; numbers -4 and -6 are measurements during vegetation period

Yield and oil content in achenes

The yield of sunflower was more favourable in 2012 when the achieved yield was higher in comparison with 2013 (Table 1). The results of combined ANOVA showed high significant influence of year weather conditions on the yield of sunflower achenes (Table 2). The results agree well with other researches (Pereyra-Irujo & Aguirrezábal 2007; Mátyás *et al.* 2014), who confirmed high significant influence of year-round weather conditions on the yield of sunflower achenes. After evaluation of impact of hybrids on the yield, we found that the highest yield was achieved in 2013 with the NK Neoma hybrid, and the lowest in 2013 with NK Brio hybrid (Table 1). The impact of genotype on the yield of achenes was highly significant (Table 2). The results agree with the observations of Bakhat *et al*. (2006), Ibrahim (2012) and Mátyás *et al.* (2014) who confirmed a significant influence of genotype on yield of sunflower achenes. After influence assessment of foliar preparations on the yield, we found that the highest yield was in variant with Terra-Sorb® Foliar preparation application in 2012 with NK Brio hybrid (Table 1). This finding does not confirm to Mátyás *et al.* (2014) who recorded higher yield in control variant. The lowest yield was found in variant with Unicum® preparation application in 2013 with NK Brio hybrid (Table 1). In our experiments, we found high significant influence of treatment on the yield of achenes (Table 2). The achieved results agree well with Černý & Veverková (2012) who stated that the impact of foliar preparations on yield of sunflower achenes was significant. On the basis of experimental results, we consider the foliar application of preparations on stands of sunflower as appropriate rationalization tool of its cultivation. In the experiment, we found a a positive correlation between the yield of achenes and the weight of head and weight of thousands achenes. As weight of head and weight of thousand achenes increases, the yield of achenes also increases (Table 3). Mátyás *et al.* (2014) found positive correlation between yield of achenes and weight of thousand achenes, but negative correlation between yield of achenes and weight of head. With the average of head, we found the negative correlation. With the increase in the head diameter was observed a decrease in the yield of achenes (Table 3). The achieved results are contrary to the studies of Beg and Aslam (1984) and Ali *et al.* (2007) who reported positive impact of head diameter on yield of achenes. We assume that this phenomenon could be influenced by two factors: first, we assumed that achenes were filled poorly, or second, it is possible that large inter-space occurred

Table 3

Correlation coefficients for production and physiological traits in three sunflower genotypes treated with two biopreparations during the seasons of 2012 and 2013

Traits	Head diameter	Weight of head	TAW	Yield	Oil content	CWSI-4	CWSI-6	Ig-4	Ig-6
Head diameter	1								
Weight of head	-0.158^{NS}	1							
TAW	-0.485^{+++}	0.788^{+++}	1						
Yield	-0.502^{+++}	0.522^{+++}	0.761^{+++}	1					
Oil content	0.444^{+++}	-0.365^{++}	-0.467^{+++}	-0.411^{++}	1				
CWSI-4	0.145^{NS}	-0.346^{+}	-0.311^{+}	-0.365^{++}	-0.119^{NS}	1			
CWSI-6	0.349^{++}	-0.534^{+++}	-0.617^{+++}	-0.654^{+++}	0.602^{+++}	0.247^{NS}	1		
Ig-4	-0.208^{NS}	0.343^{+}	0.388^{++}	0.483^{+++}	-0.015^{NS}	-0.762^{+++}	-0.440^{+++}	1	
Ig-6	-0.313^{+}	0.631^{+++}	0.742^{+++}	0.576^{+++}	-0.632^{+++}	-0.117^{NS}	-0.786^{+++}	0.360^{++}	1

Note: Grey scale indicates ^{NS} – not significant difference (white cell), $^{+}$, $^{++}$ and $^{+++}$ significant differences at level < 0.05 (light-grey), < 0.01 (medium-grey) and < 0.001 (dark-grey cell)
TWA – weight of thousand achenes; CWSI – crop water stress index; Ig – stomatal conductance index; numbers -4 and -6 are measurements during vegetation period

among the seeds. Less likely is the third possibility that it may be a combination of the two possible factors. The combined effect of genotype × treatment on yield of achenes was highly significant (Table 2). Mátyás *et al.* (2014) described significant influence of this combine effect on yield of achenes, which agrees well with our observations. The combined effects of genotype × year, treatment × year and genotype × treatment × year had a high significant impact on the yield of achenes (Table 2).

We found that 2013 was more favourable for oil content than 2012 (Table 1). The results of the combined ANOVA showed that unbalanced course of weather conditions had high a significant influence on oil content in sunflower achenes (Table 2). The significant influence of year-round weather conditions on oil content of sunflower achenes was described by Pereyra-Irujo and Aguirrezábal (2007) and Echarte *et al*. (2013). The assessment of genotype impact on oil content showed that the highest oil content was achieved in 2013 with the NK Brio hybrid, the lowest in 2012 with the NK Ferti hybrid (Table 1). The impact of genotype on oil content was highly significant (Table 2). Pereyra-Irujo and Aguirrezábal (2007), Gesch and Johnson (2013) and Yasin *et al*. (2013) claimed that sunflower hybrids shows differences in the oil content, which correspond with our findings. The results of combined ANOVA showed that the impact of treatment on oil content was highly significant (Table 2). Our results agree well with Černý and Veverková (2012), who found a significant influence of foliar preparations on oil content of sunflower achenes. In relation to the fat content, a positive correlation was found with the head diameter. The higher head diameter also increased the fat content of achenes (Table 3). In our experiments,oil content had a negative correlation to the weight of head, weight of thousand achenes and the yield. With decreasing weight of head value, weight of thousands achenes and the yield of achenes, the oil content increased (Table 3). The results do not confirm of the findings of Mátyás *et al.* (2014) who found a positive correlation of oil content with the weight of thousand achenes and yield of achenes. The quantitative relationship between sunflower yield and quality of production ($r_p = –0.41$, $P < 0.01$) is shown in Figure 2. However, the head diameter and weight of head has negative correlation, as reported by the author. All combined effects (G × T, G × Y, T × Y and G × T × Y) had high significant impact on oil content (Table 2).

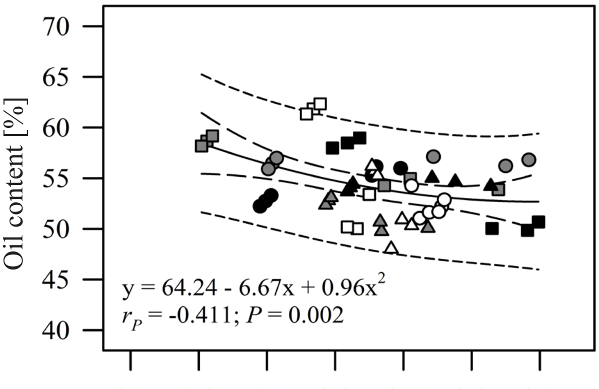

Figure 2. The relationship between yield of achenes [t/ha] and oil content [%] in three sunflower genotypes. The relationship represented two growing seasons and application of bio-preparations.
Symbols: NK Brio – square; NK Neoma – circle; NK Ferti – triangle; untreated plants – white; Terra-Sorb® Foliar – black; Unicum® – gray. Solid line shows polynomial quadratic regression, long-dash line shows 95% confidence band and short-dash line 95% prediction band.

Temperature indices

Sunflower productivity and oil content in achenes are strongly affected by the availability of soil water (Reddy *et al*. 2003; Rauf 2008) although sunflower plants are commonly regarded as drought-tolerant (Baldini *et al*. 1997). Nevertheless, many studies showed that water deficit decreased the production performance of sunflower. The yield reduction of sunflower by water shortage depends on the growth stage of the plant as well genetically determined resistance and stress severity (Langeroodi *et al*. 2014). The most critical period for water availability is reproductive stages of flowering and achenes filling (Karam *et al*. 2007; Rauf 2008). This is the main reason why we evaluated the water status during two different stages of sunflower ontogeny using infrared thermography. The reduction in the rate of transpiration, caused by inhibition of stomatal opening under drought condition, causes an increase of the crop surface temperature. The leaf surface with intensive transpiration is characterised by a lower temperature than the surrounding environment.

In agro-biological research, as well as in practical agronomy applications, the most widespread expression of these differences is the calculation of the temperature difference ($\Delta T = T^{leaf} - T^{air}$). Because ΔT (in many studies referred as canopy temperature depression [CTD]) is directly influenced by many physiological processes of plants, it is a good indicator of biological fitness genotype in a given environment.

In our study, the temperature indices were calculated in two principal yield-forming stages for sunflower (flowering and ripening). Sunflower hybrids differed significantly in crop water stress index (CWSI) and calculated stomatal conductance index (Ig) (Tables 1 and 2). The mean value of crop water stress index (CWSI) was 0.59±0.19 and significantly smaller at 2012, mainly in flowering (CWSI-4). The sum of precipitation in flowering period was 243.4 mm in 2012 and 154.2 mm in 2013, respectively. Then, the redistribution of precipitation in both years of growth period flowering and harvesting was very different. As shown in Figure 1, July and

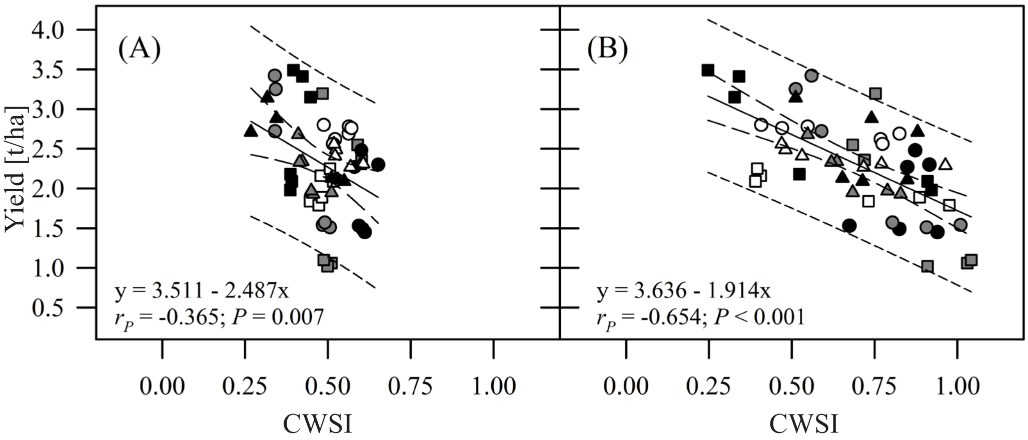

Figure 3. The relationships between yield of achenes [t/ha] and crop water stress index (CWSI) in three sunflower genotypes. CWSI was measured at BBCH 63 (A) and 87 (B). Relationships represented two growing seasons and application of bio-preparations. Symbols: NK Brio – square; NK Neoma – circle; NK Ferti – triangle; untreated plants – white; Terra-Sorb® Foliar – black; Unicum® – gray. Solid line shows linear quadratic regression, long-dash line shows 95% confidence band and short-dash line 95% prediction band.

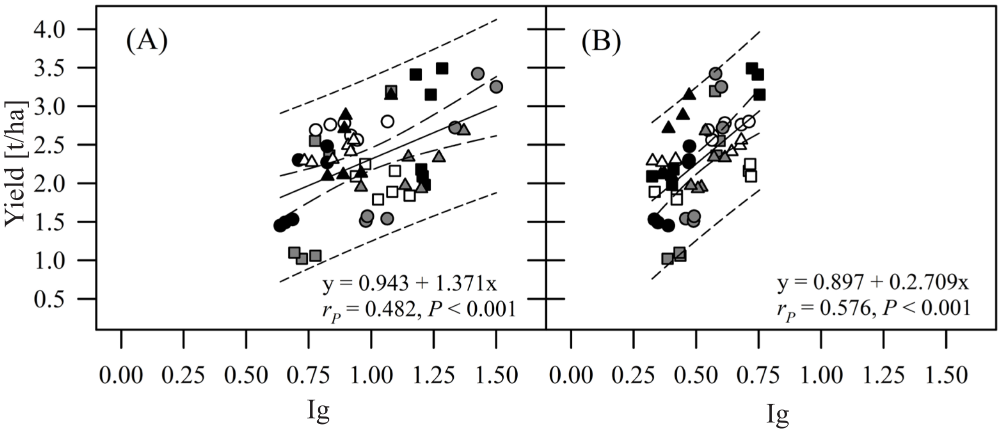

Figure 4. The relationships between yield of achenes [t/ha] and index of stomatal conductance (Ig) in three sunflower genotypes treated with biologically active substances. Ig was measured at BBCH 63 (A) and 87 (B). Relationships represented two growing seasons and application of bio-preparations. Symbols: NK Brio – square; NK Neoma – circle; NK Ferti – triangle; untreated plants – white; Terra-Sorb® Foliar – black; Unicum® – gray. Solid line shows linear quadratic regression, long-dash line shows 95% confidence band and short-dash line 95% prediction band.

August in 2013 was characterised by rainless period with higher average daily temperature, as compared with 2012. Thus, rainless period in 2013 reflects an increase of water deficit in plants and water stress as documented by the CWSI increase. During ripening of achenes (August and beginning in September 2012), the significant ($P < 0.01$) increase of CWSI was observed as a result of water deficit in both years. Table 1 shows that genotype NK Brio reached the lowest value of CWSI in both the experimental years, indicating that this genotype is more tolerant to drought than NK Neoma and NK Ferti. It was observed that foliar application of BAPs leads to a decrease of CWSI and therefore reduced water stress only in the earlier growth period. This prevention effect to water stress was evident using biopreparate Unicum®. Despite strong dependency of temperature indices on environmental factors (soil and plant water content, air temperature and humidity, wind velocity; Jones 1999), the hybrid differences in overall mean levels of CWSI and Ig were on some occasions highly significant ($P < 0.01$). Genotypic differences were observed in many studies with various field crops (Fischer *et al*. 1998; Padhi *et al*. 2012; Zia *et al*. 2012), including sunflower (Nielsen & Anderson 1989; Taghvaeian *et al*. 2014). The analysis of variance showed a significant effect ($P < 0.001$) of all combined effects (G × T, G × Y, T × Y and G × T × Y) on CWSI and Ig indices measured in both growth stages of sunflower plants (except CWSI-6) (Table 2).

The relationship between temperature indices and achenes yield of sunflower hybrids are demonstrated in Figures 3 and 4. The linear regression analysis between CWSI and yield for each phase of ontogeny were explained by a 13% and 42% ($P < 0.01$) of the variation in sunflower yield (Figure 3A, 3B). The tighter negative relationship was found when measuring CWSI during ripening of the seed. In this growth stage, the filling of sunflower seeds is very sensitive to stress situation (Rauf 2008). The increase of stress intensity results in stomatal closuring and so CWSI increases. It was also observed (Taghvaeian *et al*. 2014) that CWSI measured for sunflower hybrid strongly correlated with fraction of intercept light and leaf area index and thus the plant growth. Similar results for sunflower were observed previously by Connor *et al*. (1985). In our study, the reduced seed filling activity under ripening led to a decline of TAW and this is documented by high correlation coefficient with CWSI-6 (r_p = –0.65, $P < 0.001$) (Figure 3).

The reciprocal parameter to CWSI, stomatal conductance index (Ig), is calculated. Jones (1999) showed the strong linear correlation between stomatal conductance and I_4 index (also called as Ig), and we used this parameter for the determination of its relationship to yield of sunflower hybrids (Figure 4A, 4B). We found a strong ($P < 0.001$) positive correlation of Ig to yield (r_p = 0.48 and 0.57 for phase flowering and ripening, respectively). The maximal observed level of Ig was about 3.5, which agrees with detected maximal stomatal conductance measured for well-watered leaves of many plants (Jones 1999; Jones *et al*. 2009; Leinonen *et al*. 2006). With decreasing Ig value, a reduction in stomatal conductance is seen and thus occurrence of stressful situation resulted in yield limitation.

CONCLUSIONS

The present study shows that foliar applications of biologically active preparations (BAPs) positively influences the production performance as well oil content in seeds of selected sunflower hybrids at two meteorologically different years. The results showed that the application of selected BAPs significantly increased of sunflower yield, in particular through increase the weight of thousand seeds (r_p = 0.761, $P < 0.001$). Similarly, oil content in achenes was significantly higher in treatments with BAPs, mainly with the preparation containing free amino acids. Analysis of indices derived from infrared (IR) thermography confirmed that BAPs (containing mainly free amino acids) significantly reduced the sensitivity of stomata to stressful environmental situations (lower CWSI and higher Ig). Therefore, this response allows better plant water use and carbon storage under drought and temperature stresses, resulting in higher yield of achenes. Finally, the data obtained from IR thermography can be used for monitoring of physiological health of sunflower plants, as well in potential prediction and control of the yield.

Acknowledgements. This research was supported by the Scientific Grant Agency of Ministry of Education, Science, Research and Sport of the Slovak Republic, project no. 1/0093/13.

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Received: November 23, 2015