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The Effect of Climate, Nitrogen and Micronutrients Application on Oiliness and Fatty Acid Composition of Sunflower Achenes

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Abstract: Even though the sunflower is the second most widespread oil plant in the Czech Republic, there is a lack of information about the effects of climate, nitrogen and micronutrients application on sunflower oiliness and fatty acid composition of sunflower achenes. To obtain such information, we established five year experiment (2008–2012) to study the effect of climate, nitrogen (C-control, N 60–60 kg N ha^{-1}, N 90–90 kg N ha^{-1}, N 120–120 kg N ha^{-1}) and of foliar application of boron (N 90 + B), zinc (N 90 + Zn) and molybdenum (N 90 + Mo) on sunflower oiliness and composition of fatty acids (palmitic, palmitoleic, stearic, oleic and linoleic acids). According to our results, oiliness and fatty acid composition was significantly influenced by climate and fertilizer treatment. Oiliness was influenced mainly by climate (96.4%), the effect of fertilizer treatment was minor (2.5%). Within the frame of climate, the effect of precipitation was

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slightly higher than of temperature. A strong and negative relationship between the dose of nitrogen and oiliness was revealed ($r = -0.79$), the difference between C and N 120 treatment was 2.1% on behalf of C treatment. The highest oiliness was recorded in 2011 (50.72%) and in C treatment (48.48%). The fatty acid composition was not significantly influenced by fertilizer treatment, but was significantly influenced by the climate conditions of the year. Application of micronutrients was not connected with any significant increase in oiliness or in the fatty acid composition due to a high initial content of those micronutrients in top soil.

Keywords: boron, fatty acids, molybdenum, nitrogen, oil content, zinc

Introduction

Due to its constitution and properties, the sunflower oil is one of the most widely used vegetable oil over the world. The mean worldwide production is approximately 12,614 thousand of metric tons per year (2007–2011, National Sunflower Association), which is the fourth highest position among all vegetable oils. The sunflower oil is particularly rich in linoleic (18:2) and oleic (18:1) acids, both of which account for about 90% of the total fatty acids of triacylglycerols (Anastasi *et al.*, 2010). The consumption of unsaturated fatty acids is proclaimed as a benefit in human nutrition generally, especially decreasing the risk of coronary heart disease, stroke and cardiovascular disease (Bemelmans *et al.*, 2002; Bucher *et al.*, 2002; Kris-Etherton *et al.*, 2003). Though, several meta-analysis disclaimed the positive association between intake of saturated fatty acid/increased or intake of unsaturated fatty acid/decreased risk of cardiovascular diseases (Pietinen *et al.*, 1997; Siri-Tarino *et al.*, 2010) or of prostate cancer (Carleton *et al.*, 2013).

According to the Codex Standard for Named Vegetable Oils (2009), three sunflower types can be found now-a-day. First is a mid-oleic type (NuSun®), containing approximately 43.1–71.8% of oleic acid and 18.7–45.3% of linoleic acid. The second is a high oleic type with average content of 75–90.7% of oleic acid and third is a high linoleic type with typical containing of 48.3–74% linoleic acid and 14–39.4% of oleic acid. The National Sunflower Association (2013) mentions even fourth, high starch/high oleic type, containing on average 72% of oleic acid, 18% of stearic acid and 5% of other saturates. This last type of oil was developed through traditional breeding methods and the oil, produced from its achenes, is called Nutrisun™.

The sunflower oiliness and fatty acid profile are affected by achenes moisture (Gesch and Johnson, 2013), used hybrid (Zheljazkov *et al.*, 2011; Balalić *et al.*, 2012; Ferfuia *et al.*, 2012), location of planting (Zheljazkov *et al.*, 2011; Gesch and Johnson, 2013) and sequence of crop species within a crop rotation (Rathke *et al.*, 2005). As the oiliness decreases from the outer to the central part, the position of achenes on the capitulum is also affecting the oil content (Hassan *et al.*, 2011; Gesch and Johnson, 2013). Sowing date (Zheljazkov *et al.*, 2011; Balalić *et al.*, 2012; Ferfuia *et al.*, 2012) and water supply regime (Anastasi *et al.*, 2010) can also significantly influence sunflower oil composition. Among all, the environmental conditions, such as temperature, during the grain filling period play a crucial role in fatty acid composition (Izquierdo *et al.*, 2002).

Following the rapeseed, sunflower is the second most widespread oil plant in the Czech Republic, covering more than 20,000 ha. Czech Republic also represents the northern border of sunflower planting. Deeper knowledge of the effect of fertilizer treatment and of climate conditions on acid fatty composition can help to improve oil quality. Following the rapeseed, sunflower is the second most widespread oil plant in the Czech Republic, covering more than 20,000 ha. Czech Republic also represents the northern border of sunflower planting. Fertilization is assumed as a significant factor regulating crop yield and quality, sunflower inclusive. To obtain information on the effects of fertilization on oiliness of achenes and acid composition in productive conditions of Czech Republic, we established a five year experiment focused on the effects climate, different nitrogen rates and micronutrients application on oiliness and fatty acid composition of high linoleic sunflower hybrid ES Biba.

Materials and methods

Site description

The Čáslav experimental station is situated in the central part of the Czech Republic (49°53′29″N, 15°23′42″W). The altitude of the station is 263 m a.s.l., the average annual precipitation is 555 mm and the mean annual temperature is 8.9 °C (1956–2006, meteorological station Filipov). Soil is Greyic Phaeozem, developed on loess, with a 40–50 cm thick humus horizon. According to the Czech national classification, the experimental station belongs to the sugar beet growing area. Chemical properties of soil are given in Tables 1–3. Weather conditions of each season are given in Figure 1.

Table 1: Comparison of soil chemical properties (NH_4–N, NO_3–N and plant available (Mehlich III) P mg kg^{-1}) at the depths 0–30 cm and 30–60 cm in spring and autumn of particular years (2008–2012). Assessment of the P concentrations was done according to Budňáková *et al.* (2004).

Year	NH_4^+–N (mg kg^{-1})		NO_3–N (mg kg^{-1})		$N_{inorg.}$ (mg kg^{-1})		P (mg kg^{-1})			
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	P assessment	Autumn	P assessment
2008										
0–30	3.97	1.91	8.54	5.26	12.51	7.18	94	Good	n.a.	n.a.
30–60	0.36	1.12	6.14	1.84	6.5	2.96	18	Low	n.a.	n.a.
2009										
0–30	2.28	1.52	2.69	5.52	4.97	7.04	126	High	140	High
30–60	0.35	0.79	4.17	0.97	4.52	1.75	n.a.	n.a.	n.a.	n.a.
2010										
0–30	1.92	1.06	3.13	1.54	5.05	2.6	93	Good	74	Suitable
30–60	1.58	0.74	5.56	0.66	7.14	1.37	19	Low	12	Low
2011										
0–30	0.87	0.42	5.12	5.86	5.98	6.28	85	Good	116	High
30–60	0.59	0.24	5.49	2.22	6.08	2.46	18	Low	20	Low
2012										
0–30	1.09	1.41	7.35	3.57	8.44	4.99	84	Good	80	Suitable
30–60	1.1	1.73	5.91	2.69	7.01	4.42	23	Low	10	Low

Note: n.a.: not available.

Table 2: Comparison of soil chemical properties (concentrations of plant available (Mehlich III) K, Mg, Ca mg kg^{-1}, pH) at the depths 0–30 cm and 30–60 cm before sowing (Spring) and after harvesting (Autumn) in each year (2008–2012). Assessment of the K and Mg concentrations was done according to Budňáková *et al*. (2004).

Year	K (mg kg^{-1})				Mg (mg kg^{-1})				pH ($CaCl_2$)	
	Spring	K assessment	Autumn	K assessment	Spring	Mg assessment	Autumn	Mg assessment	Spring	Autumn
2008										
0–30	230	Good	n.a.	n.a.	158	Suitable	n.a.	n.a.	6.87	n.a.
30–60	140	Suitable	n.a.	n.a.	206	Good	n.a.	n.a.	7.04	n.a.
2009										
0–30	153	Suitable	296	Good	278	High	157	Suitable	7.02	6.87
30–60	191	Good	179	Good	158	Suitable	188	Good	7.44	7.3
2010										
0–30	241	Good	201	Good	164	Good	147	Suitable	6.44	6.36
30–60	154	Suitable	95	Low	196	Good	197	Good	6.13	6.12
2011										
0–30	209	Good	250	Good	205	Good	207	Good	6.39	6.07
30–60	141	Suitable	169	Suitable	254	Good	268	High	6.24	6.4
2012										
0–30	270	Good	200	Good	172	Good	166	Good	7.21	7.08
30–60	164	Suitable	117	Suitable	206	Good	193	Good	7.4	7.47

Note: n.a.: not available.

Table 3: Pseudo-total concentrations of boron (B), zinc (Zn) and molybdenum (Mo) in 0–30 cm upper soil layer (mg kg^{-1}) in samples collected in April.

Year/Micronutrient	2008	2009	2010	2011	2012
B	68.55	84.62	74.07	73.04	82.63
Zn	56.53	63.89	58.19	56.28	63.97
Mo	0.23	0.11	0.19	0.27	0.24

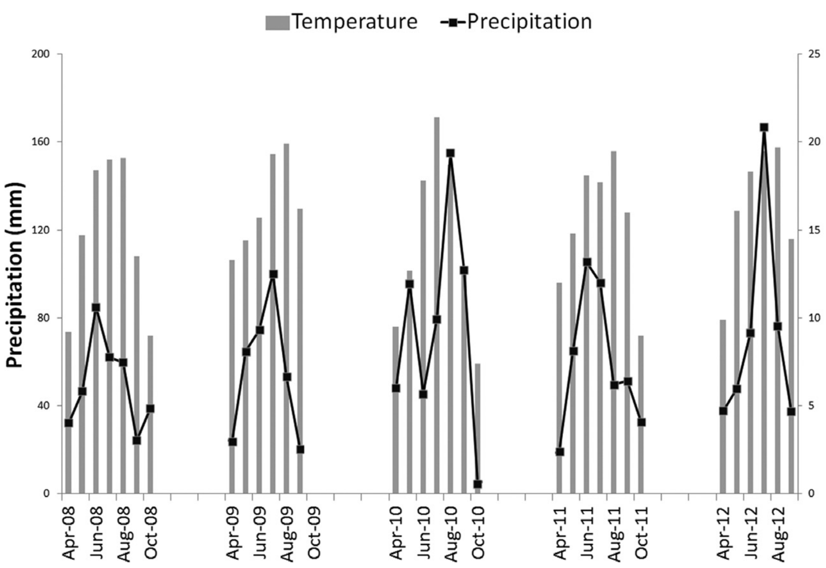

Figure 1: Temperature (°C) and precipitation (mm) during the experiment.

Soil analysis

The concentration of nitrogen (NH_4^+-N and NO_3^--N) was analysed colorimetrically (Skalar Ins.) through the potassium sulfate. Concentrations of plant available phosphorus, potassium, magnesium and calcium were extracted by Mehlich-3 solution (Mehlich, 1984) and then determined by ICP-OES (TraceScan, Thermo Jarrel Ash, Franklin, USA). The pH value was analysed in $CaCl_2$ solution according to ČSN ISO 10390 (836221). Pseudo-total concentrations of boron, molybdenum and zinc were analysed by microwave decomposition in the *aqua regia* and then determined by spectrometer (TraceScan, Thermo Jarrel Ash, Franklin, USA).

Experimental design

The experiment was established in 2008 and finished in 2012. The sunflower hybrid ES Biba, early maturing with high yields and high oiliness, was sown on different field each year with cereals as preceding crop. The harvest of cereals was in autumn followed by stubble ploughing and 0.2 m deep tillage. Sunflower was sown in the second decade of April at the rate of 75,200 germinable achenes per ha and harvested in the first decade of October. The experiment was set in randomized complete block design with four replications. The size of experimental strip was 12 × 2.8 m, but only the central area 10 × 1.4 m was used for sample collection. The effect of seven fertilizer treatments was analysed: the control (C) without any fertilizer inputs, three different nitrogen rates (N 60–60 kg N ha^{-1}, N 90–90 kg N ha^{-1}, N 120–120 kg N ha^{-1}) and three nitrogen rates accompanied with micronutrients (N 90 + B – 0.3 kg B ha^{-1}, N 90 + Zn – 0.35 kg Zn ha^{-1}, N 90 + Mo – 0.125 kg Mo ha^{-1}). Nitrogen was applied as calcium ammonium nitrate. Micronutrients were foliarly applied as: B-boretanolamin, Zn-mixture of ZnO and $ZnSO_4$, Mo-Na_2MoO_4. Doses of nitrogen were divided into two dressing (the first dressing was applied in April before seeding and the second dressing in the second decade of June). Micronutrients were applied in June (BBCH 30–31). To provide the sufficient pool of other macronutrients, P, K and Mg were also applied. The doses of P, K and Mg were established with regard to their soil available supply and crop demand. The soil was analysed in April, before sowing, and in October, after harvest. Concrete doses of P, K and Mg applied in particular years are given in Table 4. P was applied as triple superphosphate, K was applied as KCl and Mg as kieserite. Pesticides were applied in April (Dursban 10 G, Racer 25 EC, Trophy 45, Wing P) and June (Pictor, Trophy 45).

Table 4: Doses of phosphorus (P), potassium (K) and magnesium (Mg) (kg ha^{-1}) applied to sunflower in particular years.

Element/Year	2008	2009	2010	2011	2012
P	0	0	0	30	30
K	120	100	120	170	150
Mg	40	30	40	60	50

Analysis of achene properties

The oiliness was analysed by a method using pulsed nuclear magnetic resonance spectrometry (Bruker Minispeq, mq series of TD-NMR system) according to ČSN ISO 10565:1998. Concentration of fatty acids was analysed by gas chromatography (Agilent Technologies 6890N with capillary column HP-INNOWAX) according to ISO 5508:1990.

Data analysis

All statistical analyses were performed using STATISTICA 12.0 software (www.StatSoft.com). Effect of treatment, year and treatment*year was analysed by factorial ANOVA. The effect of fertilizer treatment in a particular year was analysed by one-way ANOVA. After obtaining significant ANOVA results, a Tukey HSD post hoc test was applied to determine significant differences among individual treatments and years. Polynomial regression was used to evaluate and show the relationship between achene yield and oiliness, palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid concentrations. To analyse all data together, we used the standardized principal component analysis (PCA) in STATISTICA 12.0 software.

Results

According to factorial ANOVA, the oiliness was significantly affected by year (d.f. $= 4$, $F = 129.7$, $p < 0.001$) and fertilizer treatment (d.f. $= 6$, $F = 3.4$, $p < 0.01$). Influence of year*fertilizer treatment interaction was not statistically significant (d.f. $= 24$, $F = 0.4$, $p = 0.998$). The highest oiliness was recorded in 2011 (50.72%), the lowest in 2008 (40.25%). According to fertilizer treatments, the oiliness varied from 48.48% (C) to 46.37% (N 60) (Table 5). Comparing all nitrogen treatments, the oiliness was slightly higher in B and Mo treatments, but the differences were not statistically significant.

Concentration of palmitic acid was significantly influenced only by year (d.f. $= 4$, $F = 7.23$, $p < 0.001$). The effect of fertilizer treatment and year*fertilizer treatment interaction was not significant (d.f. $= 6$, $F = 1.62$, $p = 0.15$; d.f. $= 24$, $F = 1.14$, $p = 0.31$ respectively). The highest concentration of palmitic acid was recorded in 2008 (6.55%), the lowest in 2012 (5.93%). According to fertilizer treatments, concentration of palmitic acid varied from 6.36% (C) to 6.01%

Table 5: Oiliness (%) and concentration of palmitic acid (%) in sunflower achenes as affected by year (2008–2012) and fertilizer treatment (C, N 60, N 90, N 90 + B, N 90 + Zn, N 90 +Mo, N 120).

Fertilizer treatment/year	2008	2009	2010	2011	2012	Mean of fertilizer treatment
Oiliness (%)						
C	41.87 ± 0.54^{Aa}	49.5 ± 1.33^{Abc}	51.00 ± 0.53^{Abc}	52.09 ± 0.45^{Ac}	47.96 ± 1.28^{Ab}	48.48 ± 0.9^{B}
N 60	39.22 ± 1.24^{Aa}	47.98 ± 0.27^{Abc}	48.13 ± 1.03^{Abc}	50.85 ± 1.18^{Ac}	45.70 ± 0.42^{Ab}	46.37 ± 0.97^{A}
N 90	39.58 ± 1.74^{Aa}	46.65 ± 0.24^{Abc}	49.16 ± 0.65^{Abc}	51.04 ± 1.34^{Ac}	46.04 ± 0.2^{Ab}	46.49 ± 0.98^{A}
N 90 + B	41.16 ± 1.21^{Aa}	48.50 ± 0.7^{Ab}	49.50 ± 1.13^{Ab}	50.70 ± 1.26^{Ab}	47.13 ± 0.38^{Ab}	47.40 ± 0.86^{AB}
N 90 + Zn	39.25 ± 0.9^{Aa}	47.29 ± 0.38^{Ab}	49.20 ± 1.4^{Ab}	49.98 ± 1.3^{Ab}	47.05 ± 0.59^{Ab}	46.55 ± 0.96^{A}
N 90 + Mo	40.84 ± 1.17^{Aa}	47.77 ± 0.65^{Abc}	49.45 ± 0.61^{Ac}	50.25 ± 1.24^{Ac}	45.51 ± 0.5^{Ab}	46.76 ± 0.85^{AB}
N 120	39.82 ± 0.72^{Aa}	46.56 ± 0.66^{Ab}	48.67 ± 0.96^{Abc}	50.13 ± 1.04^{Ac}	46.71 ± 0.6^{Abc}	46.38 ± 0.87^{A}
Annual mean	40.25 ± 0.42^{a}	47.75 ± 0.3^{b}	49.3 ± 0.35^{c}	50.72 ± 0.4^{d}	46.59 ± 0.27^{b}	
Palmitic acid (%)						
C	6.93 ± 0.38^{Ab}	6.61 ± 0.15^{Aab}	6.11 ± 0.06^{Aab}	6.22 ± 0.08^{Aab}	5.91 ± 0.07^{Aa}	6.36 ± 0.11^{A}
N 60	6.82 ± 0.24^{Ab}	6.51 ± 0.2^{Aab}	6.12 ± 0.17^{Aab}	6.23 ± 0.07^{Aab}	5.84 ± 0.12^{Aa}	6.31 ± 0.1^{A}
N 90	6.37 ± 0.44^{Aa}	6.48 ± 0.31^{Aa}	6.00 ± 0.15^{Aa}	6.03 ± 0.12^{Aa}	5.82 ± 0.04^{Aa}	6.14 ± 0.12^{A}
N 90 + B	5.77 ± 0.54^{Aa}	6.38 ± 0.16^{Aa}	5.96 ± 0.11^{Aa}	6.25 ± 0.11^{Aa}	5.91 ± 0.04^{Aa}	6.05 ± 0.12^{A}
N 90 + Zn	7.03 ± 0.4^{Ab}	6.40 ± 0.21^{Aab}	5.81 ± 0.16^{Aa}	6.15 ± 0.06^{Aab}	5.91 ± 0.04^{Aa}	6.26 ± 0.13^{A}
N 90 + Mo	7.05 ± 0.25^{Ab}	6.06 ± 0.12^{Aa}	6.19 ± 0.07^{Aa}	6.18 ± 0.07^{Aa}	6.20 ± 0.22^{Aa}	6.34 ± 0.11^{A}
N 120	5.90 ± 0.84^{Aa}	6.05 ± 0.12^{Aa}	5.94 ± 0.08^{Aa}	6.22 ± 0.08^{Aa}	5.93 ± 0.22^{Aa}	6.01 ± 0.16^{A}
Annual mean	6.55 ± 0.19^{c}	6.36 ± 0.07^{bc}	6.02 ± 0.05^{ab}	6.18 ± 0.03^{ab}	5.93 ± 0.05^{a}	

Note: Means with standard errors of the mean (SE) followed by the same letter (^{A} vertically, ^{a} horizontally) were not significantly different at 0.05 probability level.

(N 120) (Table 5). Addition of micronutrients was not connected with any positive or negative influence on palmitic acid concentration.

Concentration of palmitoleic was significantly influenced by year (d.f. = 4, $F = 13.44$, $p < 0.001$) and by year*fertilizer treatment interaction (d.f. = 24, $F = 2.12$, $p < 0.01$). The effect of fertilizer treatment was not significant (d.f. = 6, $F = 1.84$, $p = 0.098$). The highest concentration of palmitoleic acid was recorded in 2008 (0.17%), the lowest in 2010 and 2011 (0.07%). Between all fertilizer treatments, the concentration of palmitoleic acid varied from 0.12% (N 60) to 0.08% (N 90 + Mo) (Table 6). Addition of micronutrients was not connected with any positive or negative influence on palmiticoleic acid concentration.

Concentration of stearic acid was significantly influenced by year (d.f. = 4, $F = 62.75$, $p < 0.001$) and by fertilizer treatment (d.f. = 6, $F = 3.53$, $p < 0.01$). No effect of year*fertilizer treatment interaction was recorded (d.f. = 24, $F = 0.89$, $p = 0.62$). The highest concentration was recorded in 2008 (6.24%), the lowest in 2012 (4.29%). Between all fertilizer treatments, the concentration of stearic acid ranged from 5.25 (N 90 + Zn) to 4.6 (C) (Table 6). Application of Zn and Mo increased the concentration of stearic acid significantly (together with N 120), when compared to control treatment. But studying each year separately, the only differences between treatments were recorded only in one year, 2010 (Table 6). This leads us to assume that the final differences between all treatments can't be taken on behalf of Zn or Mo, but on behalf of climate conditions.

Concentration of oleic acid was significantly influenced only by year (d.f. = 4, $F = 47.67$, $p < 0.001$). The effect of fertilizer treatment and year*fertilizer treatment interaction was not significant (d.f. = 6, $F = 0.56$, $p = 0.76$; d.f. = 24, $F = 0.38$, $p = 0.996$ respectively). The highest concentration was recorded in 2008 (27.2%), the lowest in 2011 (18.51%). Between all fertilizer treatments, the concentration of oleic acid varied from 22.63% (N 60) to 21.56 (C) (Table 7). Addition of micronutrients was not connected with any positive or negative influence on oleic acid concentration.

Concentration of linoleic acid was significantly influenced by year (d.f. = 4, $F = 60.79$, $p < 0.001$), while the effect of fertilizer treatment and year*fertilizer treatment interaction was not (d.f. = 6, $F = 0.99$, $p = 0.44$; d.f. = 24, $F = 0.43$, $p = 0.991$ respectively). The highest concentration was recorded in 2011 (70.82%), the lowest in 2008 (59.77%). The highest concentration was provided by C treatment (67.38%), the lowest by N 90 + Mo (65.79%) (Table 7). Addition of micronutrients was not connected with any positive or negative influence on linoleic acid concentration.

Table 6: concentration of palmitoleic and stearic acids (%) in sunflower achenes as affected by year (2008–2012) and fertilizer treatment (C, N 60, N 90, N 90 + B, N 90 + Zn, N 90 +Mo, N 120).

Fertilizer treatment/Year	2008	2009	2010	2011	2012	Mean of fertilizer treatment
Palmitoleic acid (%)						
C	0.13 ± 0.03^{Aa}	0.16 ± 0.08^{Aa}	0.07 ± 0.01^{Aa}	0.08 ± 0.01^{Aa}	0.07 ± 0.01^{Aa}	0.10 ± 0.02^{A}
N 60	0.27 ± 0.09^{Ab}	0.13 ± 0.04^{Aab}	0.06 ± 0.01^{Aa}	0.08 ± 0.01^{Aa}	0.08 ± 0.01^{Aab}	0.12 ± 0.02^{A}
N 90	0.23 ± 0.05^{Ab}	0.08 ± 0.01^{Aa}	0.07 ± 0.01^{Aa}	0.07 ± 0.01^{Aa}	0.08 ± 0.01^{Aa}	0.10 ± 0.02^{A}
N 90 + B	0.15 ± 0.05^{Aa}	0.08 ± 0.01^{Aa}	0.08 ± 0.01^{Aa}	0.07 ± 0.01^{Aa}	0.08 ± 0.01^{Aa}	0.09 ± 0.01^{A}
N 90 + Zn	0.16 ± 0.003^{Ab}	0.05 ± 0.02^{Aa}	0.07 ± 0.01^{Aa}	0.07 ± 0.01^{Aa}	0.09 ± 0.01^{Aa}	0.09 ± 0.009^{A}
N 90 + Mo	0.15 ± 0.02^{Ab}	0.08 ± 0.01^{Aa}	0.05 ± 0.01^{Aa}	0.07 ± 0.01^{Aa}	0.08 ± 0.01^{Aa}	0.08 ± 0.01^{A}
N 120	0.12 ± 0.03^{Aa}	0.09 ± 0.01^{Aa}	0.07 ± 0.01^{Aa}	0.07 ± 0.01^{Aa}	0.08 ± 0.01^{Aa}	0.09 ± 0.01^{A}
Annual mean	0.17 ± 0.02^{b}	0.10 ± 0.01^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}	
Stearic acid (%)						
C	5.44 ± 0.21^{Ab}	4.92 ± 0.39^{Aab}	4.25 ± 0.25^{Aa}	4.20 ± 0.18^{Aa}	4.18 ± 0.11^{Aa}	4.60 ± 0.15^{A}
N 60	6.30 ± 0.59^{Ab}	5.04 ± 0.16^{Aab}	4.97 ± 0.23^{ABa}	4.54 ± 0.04^{Aa}	4.26 ± 0.08^{Aa}	5.02 ± 0.2^{AB}
N 90	6.47 ± 0.53^{Ab}	5.27 ± 0.18^{Aa}	4.86 ± 0.07^{ABa}	4.38 ± 0.06^{Aa}	4.39 ± 0.11^{Aa}	5.07 ± 0.2^{AB}
N 90 + B	5.99 ± 0.08^{Ad}	5.36 ± 0.09^{Ac}	4.85 ± 0.07^{ABb}	4.41 ± 0.08^{Aa}	4.17 ± 0.14^{Aa}	4.96 ± 0.16^{AB}
N 90 + Zn	6.94 ± 0.31^{Ac}	5.44 ± 0.32^{Ab}	5.07 ± 0.2^{Bab}	4.40 ± 0.1^{Aa}	4.38 ± 0.13^{Aa}	5.25 ± 0.23^{B}
N 90 + Mo	6.58 ± 0.85^{Ab}	5.99 ± 0.24^{Aab}	4.86 ± 0.14^{ABab}	4.35 ± 0.01^{Aa}	4.32 ± 0.1^{Aa}	5.22 ± 0.26^{B}
N 120	5.99 ± 0.52^{Ab}	5.77 ± 0.12^{Ab}	5.27 ± 0.12^{Bab}	4.60 ± 0.06^{Aa}	4.34 ± 0.08^{Aa}	5.19 ± 0.18^{B}
Annual mean	6.24 ± 0.19^{d}	5.4 ± 0.1^{c}	4.88 ± 0.08^{b}	4.41 ± 0.04^{a}	4.29 ± 0.04^{a}	

Note: Means with standard errors of the mean (SE) followed by the same letter (^{A} vertically, ^{a} horizontally) were not significantly different at 0.05 probability level.

Table 7: concentration of oleic and linoleic acids (%) in sunflower achenes as affected by year (2008–2012) and fertilizer treatment (C, N 60, N 90, N 90 + B, N 90 + Zn, N 90 +Mo, N 120).

Fertilizer treatment/Year	2008	2009	2010	2011	2012	Mean of fertilizer treatment
Oleic acid (%)						
C	24.69 ± 1.29^{Ab}	21.47 ± 2.23^{Aab}	19.32 ± 0.43^{Aab}	18.61 ± 0.48^{Aa}	23.72 ± 1.52^{Aab}	21.56 ± 0.77^{A}
N 60	27.86 ± 2.1^{Ab}	22.26 ± 2.03^{Aab}	20.37 ± 0.77^{Aa}	18.54 ± 0.17^{Aa}	24.12 ± 0.6^{Aab}	22.63 ± 0.92^{A}
N 90	25.72 ± 0.77^{Ac}	20.82 ± 0.54^{Ab}	20.43 ± 0.26^{Aab}	18.23 ± 0.3^{Aa}	23.41 ± 0.61^{Ac}	21.72 ± 0.63^{A}
N 90 + B	26.99 ± 2.67^{Ab}	20.99 ± 1.18^{Aa}	20.64 ± 0.46^{Aa}	18.26 ± 0.22^{Aa}	23.05 ± 0.55^{Aab}	21.98 ± 0.86^{A}
N 90 + Zn	27.56 ± 2.44^{Ab}	21.62 ± 0.97^{Aa}	20.64 ± 0.41^{Aa}	18.50 ± 0.33^{Aa}	23.65 ± 0.84^{Aab}	22.4 ± 0.86^{A}
N 90 + Mo	28.83 ± 2.71^{Ab}	22.67 ± 1.96^{Aab}	19.79 ± 0.21^{Aa}	18.15 ± 0.18^{Aa}	23.38 ± 1.12^{Aab}	22.57 ± 1.05^{A}
N 120	28.71 ± 2.5^{Ab}	21.64 ± 0.82^{Aa}	20.09 ± 0.39^{Aa}	19.25 ± 0.31^{Aa}	22.59 ± 0.41^{Aa}	22.46 ± 0.9^{A}
Annual mean	27.20 ± 0.78^{d}	21.64 ± 0.52^{bc}	20.18 ± 0.17^{ab}	18.51 ± 0.12^{a}	23.42 ± 0.31^{c}	
Linoleic acid (%)						
C	62.81 ± 1.14^{Aa}	66.83 ± 2.46^{Aab}	70.24 ± 0.67^{Ab}	70.88 ± 0.54^{Ab}	66.12 ± 1.39^{Aab}	67.38 ± 0.88^{A}
N 60	58.75 ± 2.5^{Aa}	66.06 ± 1.86^{Ab}	68.48 ± 0.91^{Ab}	70.61 ± 0.21^{Ab}	65.69 ± 0.46^{Ab}	65.92 ± 1.09^{A}
N 90	61.21 ± 1.72^{Aa}	67.34 ± 0.46^{Ab}	68.64 ± 0.19^{Abc}	71.30 ± 0.38^{Ac}	66.30 ± 0.65^{Ab}	66.96 ± 0.84^{A}
N 90 + B	61.10 ± 3.12^{Aa}	67.19 ± 1.29^{Aab}	68.47 ± 0.3^{Ab}	71.02 ± 0.23^{Ab}	66.79 ± 0.47^{Aab}	66.92 ± 0.96^{A}
N 90 + Zn	57.83 ± 2.89^{Aa}	66.50 ± 1.0^{Ab}	68.41 ± 0.47^{Ab}	70.87 ± 0.37^{Ab}	65.97 ± 0.89^{Ab}	65.92 ± 1.16^{A}
N 90 + Mo	57.39 ± 2.95^{Aa}	65.20 ± 1.9^{Ab}	69.10 ± 0.29^{Ab}	71.24 ± 0.18^{Ab}	66.03 ± 1.12^{Ab}	65.79 ± 1.27^{A}
N 120	59.28 ± 2.96^{Aa}	66.45 ± 0.66^{Ab}	68.64 ± 0.48^{Ab}	69.85 ± 0.35^{Ab}	67.05 ± 0.3^{Ab}	66.25 ± 1.01^{A}
Annual mean	59.77 ± 0.93^{a}	66.51 ± 0.53^{b}	68.86 ± 0.21^{c}	70.82 ± 0.14^{c}	66.28 ± 0.29^{b}	

Note: Means with standard errors of the mean (SE) followed by the same letter (^{A} vertically, ^{a} horizontally) were not significantly different at 0.05 probability level.

Discussion

The composition of fatty acids was corresponding to the ES Biba genetic background. As it is the linoleic type of sunflower, the major part of oiliness was achieved by linoleic acid (Figure 2). Fertilization treatment significantly influenced oiliness (Figure 3a) (Table 5) and the concentration of stearic (18:0) (Figure 4(a)) acid. The relationship between oiliness and nitrogen rate applied to sunflower was found as strongly negative ($r = -0.79$), which means that with increasing rate of nitrogen the oiliness decreased. The oiliness difference between C and N 120 treatment was 2.1% on behalf of the C treatment. Similar results published Mohammadi *et al*. (2013) for sunflower or Rathke *et al*. (2005) and Mohammadi and Rokhzadi (2012) for Canola. The cause of this negative relationship consists in the inner competition between protein biosynthesis and fatty acid synthesis. These two biochemical processes are dependent on carbon

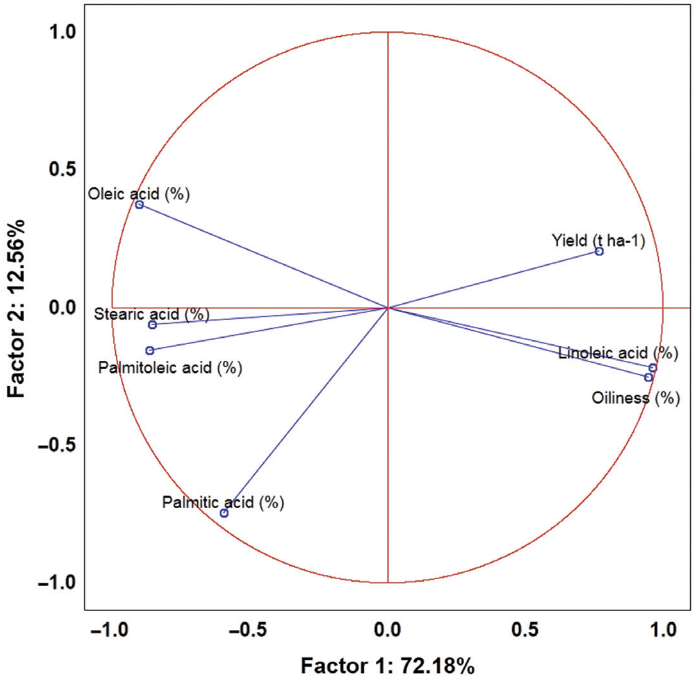

Figure 2: Ordination diagram showing the results of PCA analysis.

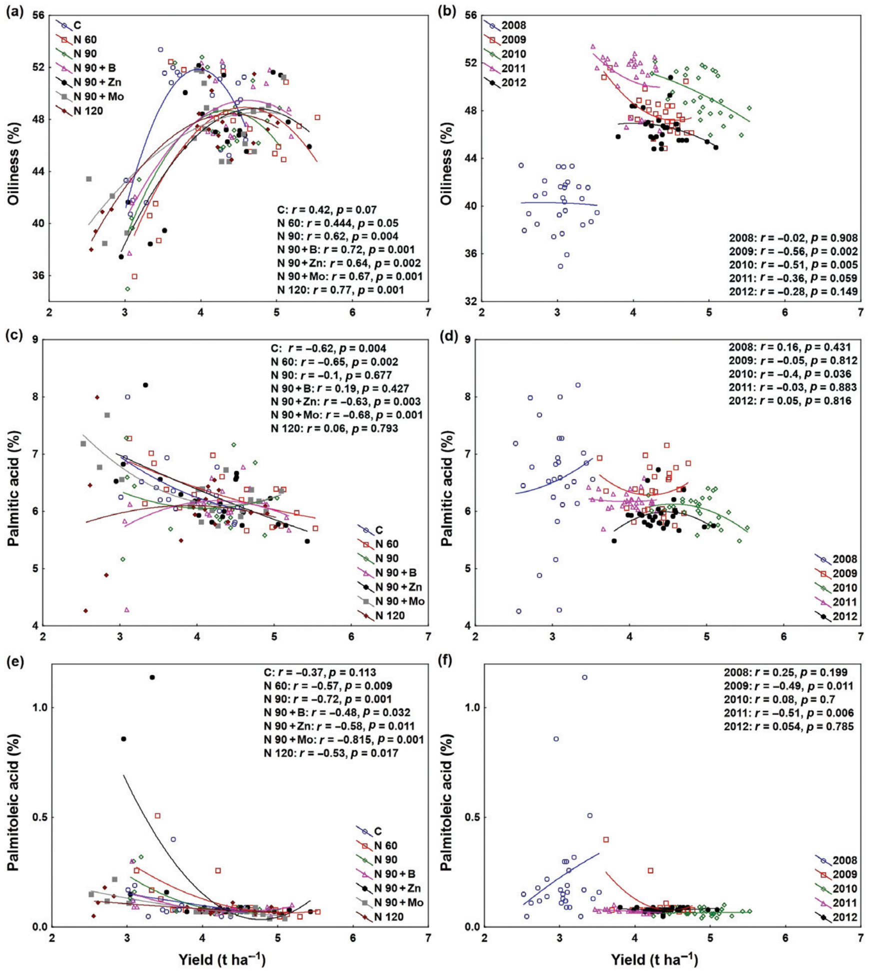

Figure 3: (a) Oiliness, (c) palmitic acid and (e) palmitoleic acid content as a function of achenes yield in fertilizer treatments (C, N 60, N 60 + B, N 60 + Mo, N 60 + Zn, N 90, N 120). (b) Oiliness, (d) palmitic acid and (f) palmitoleic acid content as a function of achene yield in the years 2008–2012.

compounds from carbohydrate metabolism (Bhatia and Rabson, 1976). As proteins require lower content of carbohydrates when compared to oils and as nitrogen fertilization leads to higher nitrogen uptake by plants, the increased nitrogen supply intensifies proteosynthesis at the expense of fatty acid synthesis, reducing the oil content of the seed. In our case, the greyic Phaeozem in Časlav experimental station represents one of the most fertile soil type in the

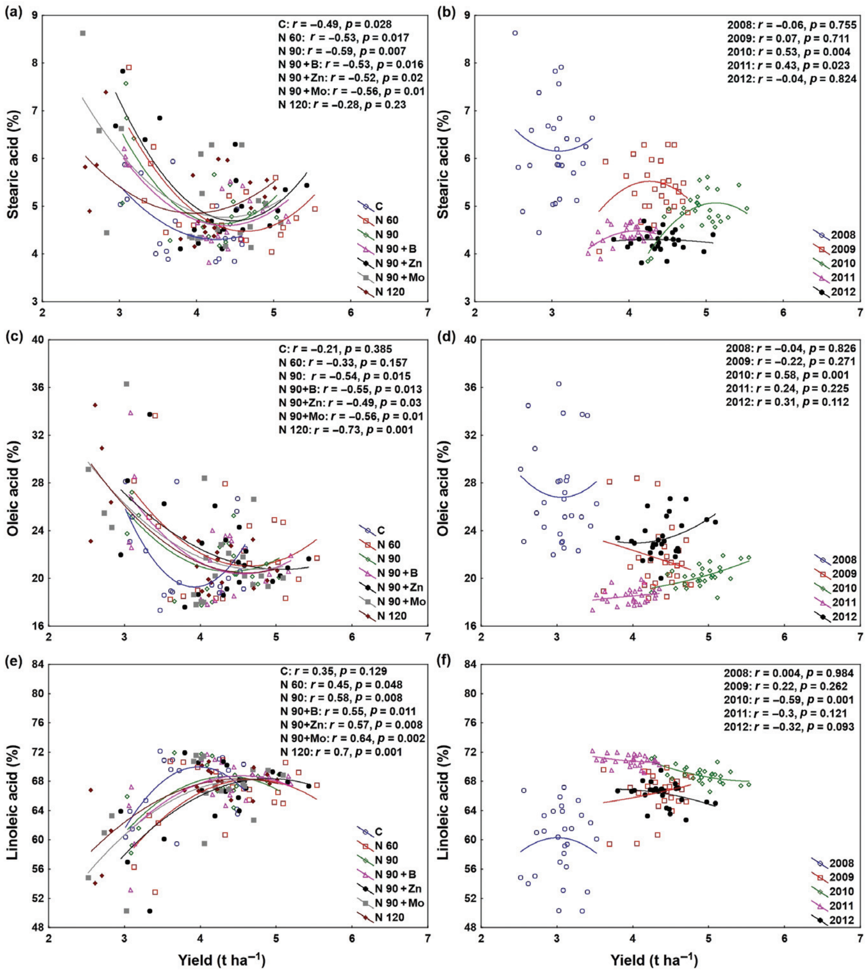

Figure 4: (a) Stearic acid, (c) oleic acid and (e) linoleic acid content as a function of achenes yield in fertilizer treatments (C, N 60, N 60 + B, N 60 + Mo, N 60 + Zn, N 90, N 120). (b) Stearic acid, (d) oleic acid and (f) linoleic acid content as a function of achene yield in the years 2008–2012.

Czech Republic (Kunzová and Hejcman, 2010) with the good initial content of nitrogen and mostly good content of other macronutrients (Tables 1 and 2). This explains the highest oiliness in C treatment and lower oiliness in the other nitrogen treatments. The oiliness was not affected by application of micronutrients. Stearic acid was slightly influenced by fertilizer treatment (Figure 4(a)). Anyway, high fluctuation between the stearic acid content, causing the statistically significant differences between fertilizer treatments, was recorded only in 2010. In this year, the concentration of stearic acid responded positively to the N 90 + Zn a N 90 + Mo and to $N + 120$ treatments. As this effect occurred only in the one of five years, we assume that some short-time combination of local disfavourable effects influenced the stearic acid content and we can't affirm this record as a wildly exercisable rule.

Much more higher fluctuation of oiliness was recorded between the particular years (Table 5) (Figure 3(b)). In fact, the factor "year" influenced the oiliness by 96.4% ("fertilizer treatment" factor by only 2.5%). Our results contradict those of Flagella *et al.* (2002) and De Giorgio *et al.* (2007), who did not record any effect of the year on oiliness in theirs two and four years long experiments. In our case, significant differences in sunflower achenes yield between particular years were also recorded (data not shown). As the yield fluctuated during the years, so did the oiliness, revealing a strong positive relationship between those two parameters ($r = 0.77$). Hlisnikovský *et al.* (2014) published a significant effect of weather on winter wheat grain yield and its protein content. In particular years, the winter wheat protein content increased rapidly as the yield decreased. Although sunflower seed protein and carbohydrate content were not measured in our study, we assume that the weather conditions of the particular year influenced protein content and oiliness, the two parameters in an inverse relationship, causing oiliness fluctuations.

Concentrations of all fatty acids were significantly influenced by climate (Figures 3(d) and 3(f). 4(b), 4(d), and 4(f)). The effect of climate, especially temperature, is known for a long time and well documented not only for sunflower. Canvin (1965) studied the oil content of several oil crops under four different temperature conditions during the period of seed development. He discovered that concentration of more highly unsaturated fatty acids decreased as the temperature increased. In his experiment, the saturated acids were not affected by temperature at all. Similar results published Sarmiento *et al.* (1998), who revealed a strong variation in the proportions of oleic and linoleic acids according to different temperature regimes, while saturated acids were affected less. Plants growing at a constant low temperature provided seeds with the highest concentration of linoleic acids, while the lowest level of linoleic acid was obtained by growing the seeds at high temperature. The positive relationship between increasing temperature and oleic acid concentration also published Ferfuia *et al.* (2012), who studied the effect of temperature on fatty acid composition of two high oleic inbred lines and hybrids. According to Sarmiento *et al.* (1998), two desaturases, induced by low and basal temperature, are operating in sunflower seeds. The basal desaturase activity is responsible for the low synthesis of linoleic acid at high temperatures and its activity increases as the temperature decrease. According to our data, we can't confirm this relationship. In fact, the opposite is true. During the years with higher mean temperature, the concentration of oleic acid slightly decreased ($r = -0.3$) and of linoleic acid slightly increased ($r = 0.34$). Relatively small values of correlation coefficients could be explained by a wide range of temperatures, especially during the grain filling period. Under Czech Republic's climate conditions, temperatures during the yield formation period can fluctuate rapidly. In our case, the temperature ranged from 1.2 to 32.5°C and covered optimal temperature areas of both above mentioned desaturate enzymes.

Conclusions

From the results obtained from 5-year field experiment with sunflower cropping follows that:

1. Fertilizer treatment influenced the oiliness, composition and concentration of fatty acids minimally. In given conditions, application of N-fertilizers had a negative correlation to the oiliness of sunflower achenes.
2. Due to the sustainable initial pool of micronutrients in the top soil layer, the foliar application of B, Zn and Mo didn't provide any significant increase in oiliness or fatty acid concentrations.
3. Significant fluctuations of temperature and precipitation significantly influenced the oiliness and sunflower fatty acid composition.

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