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Effect of Nitrogen, Boron, Zinc and Molybdenum Application on Yield of Sunflower (*Helianthus annuus* L.) on Greyic Phaeozem in the Czech Republic

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Abstract: The sunflower is a newly planted crop in the Czech Republic, which represents the northern edge of the species' range, and there is a lack of information about the effect of nutrient application on yield components. To fill this gap, we performed a fertilizer experiment on Greyic Phaeozem over the years 2008–2012 to evaluate the effects of N, B, Zn and Mo application on achenes yield. We compared the control (C) without any N fertilizer, N treatments (N 60, N 90, N120 kg N ha^{-1}) and N treatments 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}). The mean achenes yield over all years ranged from 3.9 in C to 4.34 t ha^{-1} in N 60. Achenes yield over all treatments ranged from 3.04 in 2008 to 4.91 t ha^{-1} in 2010. We concluded that sunflower can be produced with application rate up to 60 kg N ha^{-1} on highly productive soils in the Czech Republic and for similar other soils in the region. The increase in N application above 60 kg N ha^{-1} can decrease achenes yield. Application of B and Zn achieved slightly higher, while the addition of Mo slightly lower achenes yields when compared to the treatment with the same level of N and without micronutrients addition. We concluded that the application of micronutrients at the sites with sufficient content of those micronutrients in soil has almost no

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effect on achenes and biomass yield. Achenes and biomass yield was primarily affected by weather conditions in particular years, affecting plant growth, disease and predatory occurrence. Fertilizer treatments did not significantly affect the weight of achenes per head, weight of thousand achenes, the number of achenes per head, the number of heads per ha and number of achenes per ha during the experiment. The significant effect of the year was recorded, showing major effect of weather conditions on above mentioned parameters.

Keywords: sunflower, nitrogen fertilization, micronutrients, yield, biomass

Introduction

The sunflower is a valuable source of edible oil, 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 non-food utilization of sunflower finds application in agriculture as a direct feedstuff or silage material and in pharmaceutical and chemical industries. Phytomass of sunflower can also be used as a source of renewable energy via biomass burning (Alaru *et al.*, 2011).

The yield of sunflower achenes can be significantly affected by sunflower hybrid (De Giorgio *et al.*, 2007; Zheljazkov *et al.*, 2011; Ibrahim, 2012), location (Zheljazkov *et al.*, 2011), sowing date (Göksoy *et al.*, 1998; Barros *et al.*, 2004), plant density (Vijayalakshmi *et al.*, 1975; Göksoy *et al.*, 1998; Barros *et al.*, 2004; Zheljazkov *et al.*, 2011), tillage method (Aboudrare *et al.*, 2006) and by nutrient supply (Süzer, 1998; De Giorgio *et al.*, 2007; Osman and Awed, 2010; Oyinlola *et al.*, 2010; Ali *et al.*, 2012; Nasim *et al.*, 2012; Sincik *et al.*, 2013). Among the macronutrients, such as nitrogen, phosphorus and potassium, the micronutrients, such as boron, zinc and molybdenum, play a significant role in plant development and sustainable yield and quality achieving. All three elements are essential for plant growth and have their own special functions inside plant`s structures. Boron is the only non-metal element among plant micronutrients. Its deficiency rapidly inhibits root elongation and growth, cause reduction in crop yields, impair crop quality, or have both effects (Gupta, 2006). In the plant, boron is associated with water relation, sugar translocation, cation and anion absorption (Oyinlola, 2007) and adequate B supply can increase achenes yield by decreasing the sterility (Al-Amery *et al.*, 2011). Zinc is an essential part of enzyme structure and has catalytic, coactive and structural functions. Its deficiency appears as intervenial chlorosis in which lighter green to pale yellow color appears between the midrib and secondary veins (Storey, 2006). According to Alloway (2009), Zn deficiency can be exacerbated in soils by increase in P availability and by an increase in pH values. Molybdenum, similarly to Zn, is a component of more than 60 enzymes catalyzing diverse oxidation-reduction reactions (Zimmer and Mendel, 1999). It is an element needed for chemical changes associated with nitrogen nutrition and enables the plant to use nitrates taken up from the soil (Weir, 2004).

In Europe, the Czech Republic represents the northern border of sunflower planting. The sunflower is a quite newly planted crop in the Czech Republic and there is a lack of information about the effect of different N application rates and foliar application of micronutrients on achenes and aboveground biomass yield. That is why we established a fertilizer experiment on highly fertile Greyic Phaeozem in Čáslav experimental station to evaluate the effects of application of different N rates and of foliar application of B, Zn and Mo on sunflower achenes yield, weight of achenes per head, thousand achenes weight, number of achenes per head, total aboveground biomass per plant, number of heads per ha and number of achenes per ha.

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. The average content of organic carbon was 1.64% (0–30 cm), 1.09% (30–60 cm) and 0.56% (60–110 cm). The average content of nitrogen was 0.14% (0–30 cm), 0.09% (30–60 cm) and 0.06% (60–110 cm). Other soil chemical properties are given in Tables 1, 2 and 3. Weather conditions of each season are given in Figure 1.

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 spring barley as preceding crop. The harvest of spring barley (the average grain yield ranged from 4 to 4.5 t ha^{-1}) was followed by stubble ploughing and 20 cm 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 m $\times$ 2.8 m,

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

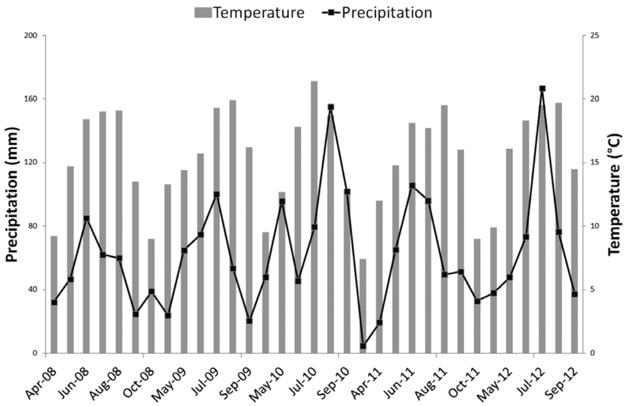

Figure 1: Average monthly temperatures (°C) and monthly sums of precipitations (mm) during the experiments.

but only the central area 10 m × 1.4 m was used for sample collection. The effect of seven fertilizer treatments were analyzed: the control (C) without any fertilizer input, three different nitrogen treatments (N 60–60 kg N ha^{-1}, N 90–90 kg N ha^{-1}, N 120–120 kg N ha^{-1}) and three treatments 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 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 on the results of the soil chemical analysis. The soil was analyzed 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 kieserit. 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

Soil analysis

The concentration of mineral nitrogen (NH_4^+-N and NO_3^--N) was analyzed colorimetrically (Skalar Ins.) in potassium sulfate extract. 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 analyzed in $CaCl_2$ solution according to ČSN ISO 10390 (836221). Pseudo-total concentrations of boron, molybdenum and zinc were analyzed by microwave decomposition in the *aqua regia* and then determined by spectrometer (TraceScan, Thermo Jarrel Ash, Franklin, USA).

Data analysis

All statistical analyses were performed using STATISTICA 12.0 software (www.StatSoft.com). Effect of treatment, year and treatment*year was analyzed by factorial ANOVA. The effect of fertilizer treatment in a particular year was analyzed 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 the relationship between grain yield and weight of achenes per head, weight of thousand achenes, number of achenes per head, total aboveground biomass of plant, number of heads per ha and number of achenes per ha.

Results

Calculated by factorial ANOVA, the achene yield was significantly influenced by fertilizer treatment (d.f. = 6, F = 12.2, $p < 0.001$), year (d. f. = 4, F = 370.5, $p < 0.001$) and treatment*year interaction (d. f. = 24, F = 4.3, $p < 0.001$).

Depending on fertilizer treatment, the mean achenes yield over all years ranged from 3.9 t ha^{-1} (C) to 4.34 t ha^{-1} (N 60). Comparing the years, mean achenes yield over all treatments ranged from 3.04 t ha^{-1} (2008) to 4.91 t ha^{-1} (2010) (Table 5).

The weight of achenes per head was not significantly influenced by fertilizer treatment (d. f. = 6, F = 1.12, p = 0.37) and by fertilizer treatment*year interaction (d. f. = 24, F = 0.999, p = 0.492). The significant effect of the year was recorded (d. f. = 4, F = 9.84, p < 0.001). Mean weight of achenes over all years ranged, according to fertilizer treatment, from 78.91 g (C) to 87.69 g (N 90 + Zn). Comparing the years, mean weight of achenes over all treatments ranged from 73.78 g (2009) to 93.74 g (2010) (Table 5). The relationship between the weight of achenes per head and yield as a function of the year and fertilizer treatment is given in Figure 2a and 2b.

The thousand achenes weight (g) was not significantly affected by fertilizer treatment (d. f. = 6, F = 2.11, p = 0.058) and by fertilizer treatment*year interaction (d. f. = 24, F = 0.93, p = 0.561). Statistically significant effect of the year was recorded (d. f. = 4, F = 55.65, p < 0.001). According to fertilizer treatment, mean thousand achenes weight over all years ranged from 59.37 g (C) to 63.01 g (N 90 + B). According to year, mean thousand achenes weight over all years ranged from 55.82 g (2011) to 71.3 g (2008) (Table 6). The relationship between the weight of thousand achenes and yield as a function of the year and fertilizer treatment are given in Figure 2c and 2d.

Number of achenes per head was not significantly affected by fertilizer treatment (d. f. = 6, F = 1.16, p = 0.35) and by fertilizer treatment*year interaction (d. f. = 24, F = 1.38, p = 0.19). The significant effect of the year was recorded (d. f. = 4, F = 14.25, p < 0.001). According to fertilizer treatment over all years, the number of achenes per head ranged from 1,322.77 (N 90 + B) to 1,437.84 (N 120) (Table 6). According to year, the number of achenes per head over all treatments ranged from 1,275.56 (2008) to 1,569.48 (2010). The relationship between the number of achenes per head and yield as a function of the year and fertilizer treatment are given in Figure 3a and 3b.

Total aboveground biomass per plant (g) was significantly influenced by fertilizer treatment (d. f. = 6, F = 2.46, p = 0.043) and by year (d. f. = 4, F = 33.03, p < 0.001) but not by fertilizer treatment*year interaction (d. f. = 24, F = 1.62, p = 0.095). According to fertilizer treatment, total aboveground biomass over all years ranged from 416.53 g (C) to 564.11 g (N 120) (Table 7). According to year, total aboveground biomass over all treatments ranged from 296.9 g (2011) to 678.08 g (2008). The relationship between the total aboveground biomass and yield as a function of the year and fertilizer treatment are given in Figure 3c and 3d.

Table 5: Yield of achenes (t ha^{-1}) and weight of achenes per head (g) as affected by fertilizer treatment (C, N 60, N 90, N 90 + B, N 90 + Zn, N 90 + Mo, N, 120) and year (2008–2012). Yield of achenes and weight of achenes per head were determined for 92% dry matter content.

Fertilizer treatment/Year	2008	2009	2010	2011	2012	Mean of fertilizer treatment
Yield of achenes (t ha^{-1})						
C	3.1 ± 0.1^{BCa}	3.9 ± 0.2^{Ab}	4.4 ± 0.1^{Ac}	3.59 ± 0.1^{Ab}	4.5 ± 0.04^{ABc}	3.90 ± 0.1^{A}
N 60	3.3 ± 0.1^{Ca}	4.4 ± 0.1^{ABbc}	5.2 ± 0.1^{Cd}	3.91 ± 0.2^{ABb}	4.8 ± 0.1^{Bcd}	4.34 ± 0.2^{E}
N 90	3.1 ± 0.03^{BCa}	4.5 ± 0.1^{Bcd}	4.8 ± 0.1^{ABd}	3.98 ± 0.1^{ABb}	4.4 ± 0.04^{Abc}	4.14 ± 0.1^{BCD}
N 90 + B	3.1 ± 0.01^{BCa}	4.5 ± 0.1^{Bc}	5.0 ± 0.1^{BCd}	4.14 ± 0.1^{Bb}	4.3 ± 0.1^{Abc}	4.21 ± 0.2^{CDE}
N 90 + Zn	3.2 ± 0.1^{Ca}	4.5 ± 0.02^{Bb}	5.2 ± 0.1^{BCc}	4.10 ± 0.1^{Bb}	4.3 ± 0.1^{Ab}	4.25 ± 0.2^{DE}
N 90 + Mo	2.8 ± 0.1^{ABa}	4.2 ± 0.1^{ABbc}	4.9 ± 0.1^{BCd}	3.98 ± 0.03^{ABb}	4.5 ± 0.1^{ABcd}	4.06 ± 0.2^{ABC}
N 120	2.7 ± 0.06^{Aa}	4.3 ± 0.2^{ABb}	4.9 ± 0.1^{BCc}	4.10 ± 0.03^{Bb}	4.1 ± 0.1^{Ab}	4.01 ± 0.2^{AB}
Annual mean	3.0 ± 0.1^{a}	4.3 ± 0.1^{c}	4.9 ± 0.1^{d}	3.97 ± 0.1^{b}	4.4 ± 0.1^{c}	
Weight of achenes per head (g)						
C	74.8 ± 2.3^{Aa}	71.9 ± 3.1^{Aa}	86.5 ± 5.2^{Aa}	89.2 ± 5.1^{Aa}	72.2 ± 2.8^{Aa}	78.9 ± 2.8^{A}
N 60	92.4 ± 8.6^{Aa}	74.9 ± 1.0^{Aa}	89.9 ± 16.6^{Aa}	86.5 ± 9.9^{Aa}	87.9 ± 8.5^{Aa}	86.3 ± 4.0^{A}
N 90	87.1 ± 8.2^{Aab}	74.7 ± 0.6^{Aa}	99.3 ± 2.0^{Ab}	$88.2 \pm \pm\ 4.6^{Aab}$	88.7 ± 0.9^{Aab}	87.6 ± 3.0^{A}
N 90 + B	99.2 ± 8.3^{Aa}	71.6 ± 1.9^{Aa}	87.0 ± 10.3^{Aa}	85.8 ± 4.8^{Aa}	78.2 ± 0.7^{Aa}	84.4 ± 3.8^{A}
N 90 + Zn	93.2 ± 0.5^{Aa}	81.0 ± 2.6^{Aa}	104.2 ± 5.7^{Aa}	90.1 ± 12.8^{Aa}	70.03 ± 14.1^{Aa}	87.7 ± 4.9^{A}
N 90 + Mo	97.9 ± 4.9^{Ab}	71.7 ± 1.1^{Aa}	98.2 ± 1.2^{Ab}	79.5 ± 5.2^{Aab}	85.9 ± 4.8^{Aab}	86.6 ± 3.7^{A}
N 120	87.4 ± 2.9^{Aa}	70.7 ± 5.2^{Aa}	91.1 ± 2.4^{Aa}	95.7 ± 8.7^{Aa}	91.1 ± 6.6^{Aa}	87.2 ± 3.5^{A}
Annual mean	90.3 ± 2.7^{bc}	73.8 ± 1.2^{a}	93.7 ± 2.8^{c}	87.8 ± 2.5^{bc}	82.0 ± 2.9^{ab}	

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.

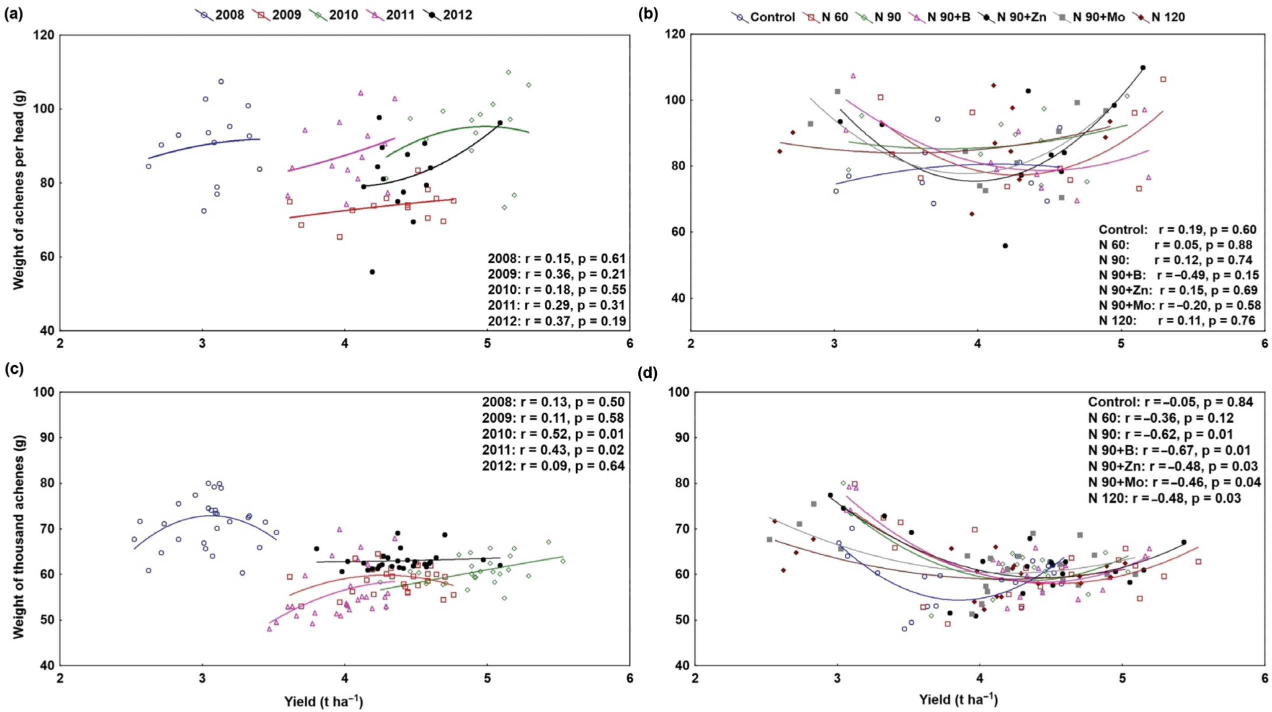

Figure 2: Relationships between the sunflower yield and its growth parameters (weight of achenes per head as affected by (a) year and (b) fertilizer treatment and weight of thousand achenes as affected by (c) year and (d) fertilizer treatment).

Table 6: Weight of thousand achenes (g) and number of achenes per head as affected by fertilizer treatment (C, N 60, N 90, N 90 + B, N 90 + Zn, N 90 + Mo, N, 120) and year (2008–2012). Weight of thousand achenes was determined for 92% dry matter content.

Fertilizer treatment/Year	2008	2009	2010	2011	2012	Mean of fertilizer treatment
Weight of thousand achenes (g)						
C	65.4 ± 21.9^{Ab}	58.9 ± 2.3^{Aab}	57.4 ± 1.6^{Aab}	52.6 ± 2.6^{Aa}	62.6 ± 0.2^{Ab}	59.4 ± 1.3^{A}
N 60	72.5 ± 2.9^{ABb}	58.7 ± 1.3^{Aa}	60.8 ± 2.4^{Aab}	56.3 ± 4.6^{Aa}	62.7 ± 0.5^{Aab}	62.2 ± 1.7^{A}
N 90	74.6 ± 1.9^{ABb}	59.1 ± 2.1^{Aa}	61.0 ± 1.4^{Aa}	55.3 ± 2.5^{Aa}	62.7 ± 0.5^{Aa}	62.5 ± 1.7^{A}
N 90 + B	76.6 ± 1.5^{Bb}	58.1 ± 1.7^{Aa}	61.5 ± 2.1^{Aa}	56.1 ± 2.5^{Aa}	62.7 ± 1.1^{Aa}	63.0 ± 1.8^{A}
N 90 + Zn	73.6 ± 1.7^{ABb}	60.7 ± 1.1^{Aa}	61.8 ± 1.9^{Aa}	56.6 ± 3.9^{Aa}	62.2 ± 0.4^{Aa}	63.0 ± 1.6^{A}
N 90 + Mo	70.1 ± 2.2^{ABc}	60.0 ± 1.6^{Aab}	60.6 ± 1.4^{Aab}	56.7 ± 2.8^{Aa}	66.1 ± 1.7^{Abc}	62.7 ± 1.4^{A}
N 120	66.3 ± 2.3^{Ab}	58.0 ± 1.4^{Aab}	60.5 ± 1.0^{Aab}	57.2 ± 3.1^{Aa}	62.4 ± 1.1^{Aab}	60.9 ± 1.1^{A}
Annual mean	71.3 ± 1.0^{d}	59.1 ± 0.6^{b}	60.5 ± 0.6^{bc}	55.8 ± 1.1^{a}	63.0 ± 0.4^{c}	
Number of achenes per head						
C	1090 ± 8^{Aa}	1277 ± 18^{Ab}	1562 ± 20^{Ac}	1582 ± 5^{Ac}	1156 ± 34^{Aa}	1333 ± 68^{A}
N 60	1331 ± 62^{Aa}	1295 ± 32^{Aa}	1561 ± 223^{Aa}	1413 ± 35^{Aa}	1421 ± 133^{Aa}	1404 ± 51^{A}
N 90	1204 ± 127^{Aa}	1336 ± 18^{Aab}	1638 ± 34^{Ab}	1530 ± 41^{Aab}	1415 ± 36^{Aab}	1424 ± 54^{A}
N 90 + B	1254 ± 107^{Aa}	1293 ± 17^{Aa}	1342 ± 175^{Aa}	1463 ± 182^{Aa}	1262 ± 1^{Aa}	1323 ± 48^{A}
N 90 + Zn	1263 ± 8^{Aa}	1375 ± 73^{Aa}	1712 ± 90^{Aa}	1449 ± 66^{Aa}	1126 ± 214^{Aa}	1385 ± 75^{A}
N 90 + Mo	1397 ± 167^{Aa}	1247 ± 43^{Aa}	1666 ± 54^{Aa}	1351 ± 32^{Aa}	1361 ± 96^{Aa}	1404 ± 56^{A}
N 120	1390 ± 3^{Aab}	1237 ± 25^{Aa}	1505 ± 69^{Aab}	1578 ± 11^{Ab}	1478 ± 100^{Aab}	1438 ± 43^{A}
Annual mean	1276 ± 38^{a}	1294 ± 16^{a}	1569 ± 45^{b}	1481 ± 31^{b}	1317 ± 47^{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.

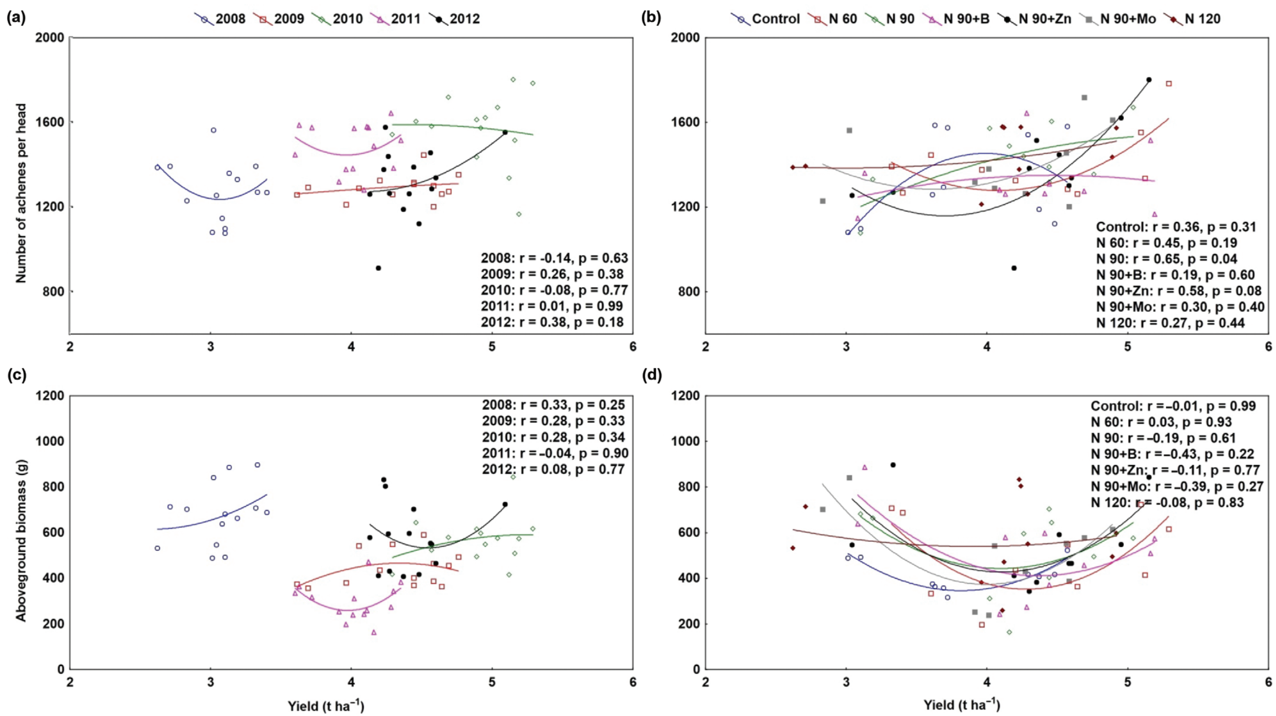

Figure 3: Relationships between the sunflower yield and its growth parameters (number of achenes per head as affected by (a) year and (b) fertilizer treatment and total aboveground biomass as affected by (c) year and (d) fertilizer treatment).

Table 7: Total aboveground biomass per plant (g) as affected by fertilizer treatment (C, N 60, N 90, N 90 + B, N 90 + Zn, N 90 + Mo, N, 120) and year (2008–2012).

Fertilizer treatment/Year	2008	2009	2010	2011	2012	Mean of fertilizer treatment
C	491.8 ± 1.8^{Ab}	366.8 ± 8.6^{Aab}	470.9 ± 52.9^{Aab}	339.7 ± 23.2^{Aa}	413.5 ± ± 4.2^{Aab}	416.5 ± 21.3^{A}
N 60	699.8 ± 10.0^{Ab}	401.7 ± 35.7^{Aab}	517.1 ± 100.7^{Aab}	266.9 ± 68.4^{Aa}	636.5 ± 88.4^{ABab}	504.4 ± 27.1^{AB}
N 90	673.2 ± 8.9^{Ab}	448.7 ± 45.5^{Aab}	610.9 ± 34.1^{Ab}	237.9 ± 73.3^{Aa}	649.4 ± 54.5^{ABb}	524.0 ± 56.7^{AB}
N 90 + B	763.4 ± 124.4^{Ab}	414.1 ± 43.1^{Aa}	542.2 ± 32.0^{Aab}	258.0 ± 15.3^{Aa}	588.9 ± 9.0^{ABab}	513.3 ± 60.1^{AB}
N 90 + Zn	722.4 ± 175.7^{Aa}	529.3 ± 63.1^{Aa}	696.0 ± 147.4^{Aa}	363.6 ± 19.7^{Aa}	439.7 ± 26.7^{Aa}	550.2 ± 58.9^{B}
N 90 + Mo	772.7 ± 68.9^{Ab}	466.1 ± 77.9^{Aab}	597.3 ± 17.4^{Ab}	246.8 ± 7.8^{Aa}	492.6 ± 61.0^{Aab}	515.1 ± 60.2^{AB}
N 120	623.2 ± 90.8^{Aab}	466.1 ± 84.8^{Aab}	547.0 ± 51.2^{Aab}	365.5 ± 106.2^{Aa}	818.7 ± 13.9^{Bb}	564.1 ± 57.2^{B}
Annual mean	678.1 ± 35.7^{d}	441.9 ± 20.8^{b}	568.8 ± 28.1^{c}	296.9 ± 21.5^{a}	577.0 ± 38.6^{cd}	

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.

The number of heads per ha was not affected by treatment (d. f. = 6, F = 1,23, p = 0,32) or treatment*year interaction (d. f. = 24, F = 1,74, p = 0,07). The significant effect of the year was recorded (d. f. = 4, F = 45.63, p < 0,001). The number of heads per ha ranged from 48,246 (N 90) to 51,831 (N 90 + B) and from 34,301 (2008) to 58,606 (2009) (Table 8). The relationship between the number of heads per ha and yield as a function of the year and fertilizer treatment are shown in Figure 4a and 4b.

The number of achenes per ha was not significantly affected by treatment (d. f. = 6, F = 0.01, p = 1), year (d. f. = 4, F = 0.95, p = 0.44) and treatment*year interaction (d. f. = 24, F = 0.01, p = 1). According to treatment, the number of achenes per ha ranged from 32,589 thousand (C) to 35,641 thousand (N 60). From 2008 to 2012, number of achenes per ha ranged from 21,656 thousand (2008) to 41,029 thousand (2010) (Table 8). The relationship between the number of achenes per ha and yield as a function of the year and fertilizer treatment are given in Figure 4c and 4d.

Discussion

Application of mineral fertilizers was accompanied with only minor increase of sunflower yield during our experiment. The highest yield was provided by N 60 treatment. Increasing of the nitrogen input above 60 kg N ha^{-1} resulted in yield decrease. Application of 120 kg N ha-1 provided the lowest yields in 2008 and 2012. These results are in agreement with Sadiq *et al.* (2000) and Ali *et al.* (2012) who also recorded negative relationship between yields and application of nitrogen in rates 100 and 150 kg N ha^{-1}. Opposite result, the positive effect of N application rates on achenes yield, were recorded generally on less fertile soils (Elhassan *et al.*, 2006; Nasim *et al.*, 2012; Sincik *et al.*, 2013). Scheiner *et al.* (2002) recorded positive response of sunflower to N application on one of two experimental sites, perhaps due to differences between soil organic matter content and shorter cropping cycles. Zubriski *et al.* (1979) evaluated the effect of seven different nitrogen rates at 23 sites over eight years. As he published, application of N increased achenes yield at 16 from the 23 sites. In those seven cases without positive reaction to N, no response of sunflower was due to poor emergence and variable plant population, due to high perched water table, due to high soil nitrate content at planting and due to other reasons. Some of these aspects appeared even in our experiment. According to ANOVA the achenes yield was influenced mainly by year (95.74%), respectively by weather conditions and other factors related to weather. Those were high emergence of hares

Table 8: Number of heads per ha and number of achenes per hectare (thousands) as affected by fertilizer treatment (C, N 60, N 90, N 90 + B, N 90 + Zn, N 90 + Mo, N, 120) and year (2008–2012).

Fertilizer treatment/Year	2008	2009	2010	2011	2012	Mean of fertilizer treatment
Number of heads per ha						
C	40 885 ± 665^{Aa}	50 891 ± 2 774^{Aab}	51 304 ± 1 482^{Aab}	41 314 ± 1 848^{Aa}	61 397 ± 3 108^{Ab}	49 158 ± 2 628^{A}
N 60	36 742 ± 3 863^{Aa}	58 992 ± 2 156^{Aa}	59 748 ± 10 084^{Aa}	44 045 ± 2 952^{Aa}	55 183 ± 2 345^{Aa}	50 942 ± 3 480^{A}
N 90	36 369 ± 2 908^{Aa}	61 548 ± 1 675^{Ac}	47 782 ± 1 979^{Ab}	46 472 ± 1 623^{Aab}	49 061 ± 1 515^{Ab}	48 246 ± 2 757^{A}
N 90 + B	31 486 ± 2 377^{Aa}	63 864 ± 3 454^{Ab}	60 377 ± 7 294^{Ab}	48 840 ± 1 596^{Aab}	54 587 ± 2 295^{Ab}	51 831 ± 4 019^{A}
N 90 + Zn	34 194 ± 1 723^{Aa}	56 212 ± 2 229^{Aa}	48 559 ± 1 701^{Aa}	48 965 ± 6 663^{Aa}	64 799 ± 10 123^{Aa}	50 546 ± 3 849^{A}
N 90 + Mo	29 919 ± 522^{Aa}	60 250 ± 4 577^{Ab}	48 815 ± 1 636^{Ab}	50 099 ± 3 915^{Ab}	51 437 ± 1 197^{Ab}	48 104 ± 3 446^{A}
N 120	30 511 ± 492^{Aa}	58 481 ± 2 000^{Ac}	53 850 ± 1 241^{Abc}	43 353 ± 3 995^{Aab}	46 733 ± 3 338^{Abc}	46 586 ± 3 320^{A}
Annual mean	34 301 ± 1 186^{a}	58 606 ± 1 332^{c}	52 919 ± 1 922^{c}	46 156 ± 1 324^{b}	54 743 ± 2 072^{c}	
Number of achenes (thousands) per ha						
C	22 271 ± 12 859^{Aa}	32 515 ± 18 861^{Aa}	40 049 ± 23 129^{Aa}	32 674 ± 18 908^{Aa}	35 435 ± 20 468^{Aa}	32 589 ± 7 724^{A}
N 60	24 327 ± 14 094^{Aa}	38 152 ± 22 030^{Aa}	45 510 ± 26 294^{Aa}	31 176 ± 18 149^{Aa}	39 041 ± 22 600^{Aa}	35 641 ± 8 528^{A}
N 90	21 711 ± 12 543^{Aa}	41 119 ± 23 780^{Aa}	39 161 ± 22 696^{Aa}	35 584 ± 20 623^{Aa}	35 685 ± 20 029^{Aa}	34 452 ± 8 223^{A}
N 90 + B	19 615 ± 11 326^{Aa}	41 260 ± 23 862^{Aa}	39 874 ± 23 023^{Aa}	35 572 ± 20 710^{Aa}	34 450 ± 19 925^{Aa}	34 154 ± 8 245^{A}
N 90 + Zn	21 607 ± 12 516^{Aa}	38 561 ± 22 267^{Aa}	41 494 ± 23 964^{Aa}	35 245 ± 20 518^{Aa}	35 400 ± 20 463^{Aa}	34 462 ± 8 229^{A}
N 90 + Mo	20 850 ± 12 163^{Aa}	37 458 ± 21 664^{Aa}	40 627 ± 23 456^{Aa}	33 894 ± 19 770^{Aa}	34 952 ± 20 224^{Aa}	33 556 ± 8 033^{A}
N 120	21 211 ± 12 248^{A}	36 149 ± 20 875^{A}	40 489 ± 23 388^{A}	34 204 ± 19 911^{A}	34 376 ± 19 847^{A}	33 286 ± 7 932^{A}
Annual mean	21 656 ± 4 195^{a}	37 888 ± 7 341^{a}	41 029 ± 7 920^{a}	34 050 ± 6 613^{a}	35 488 ± 6 848^{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.

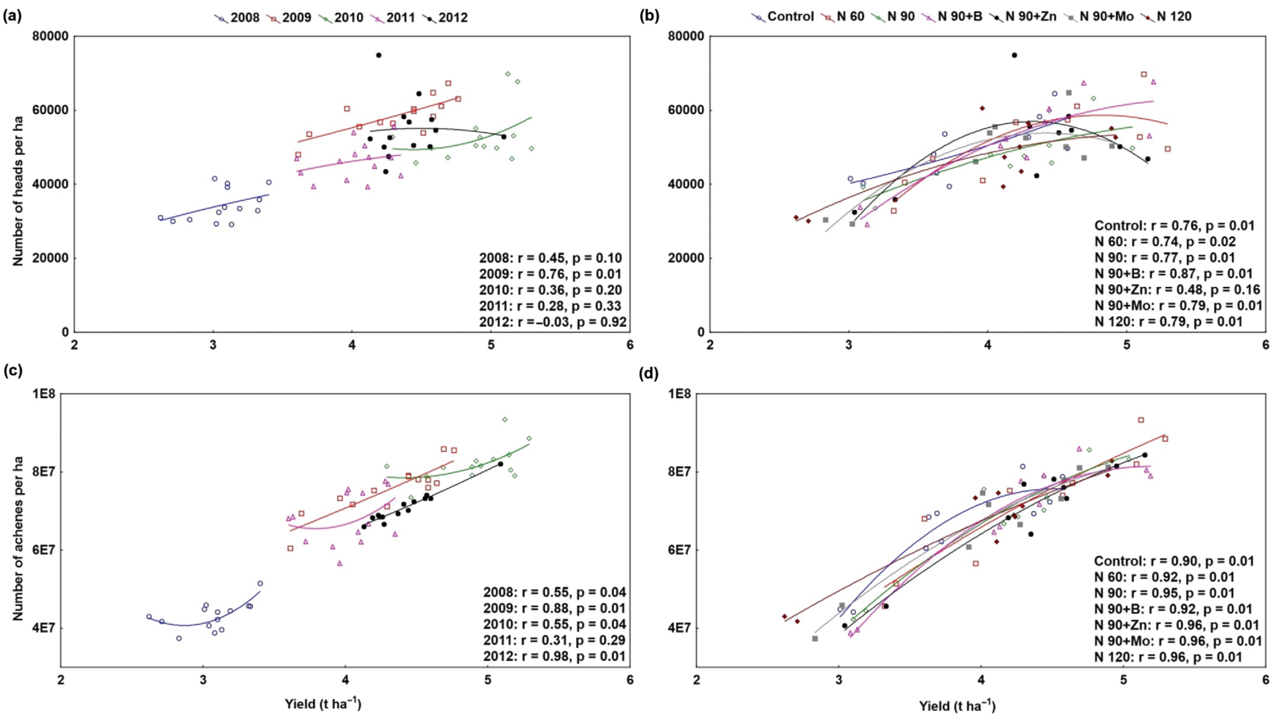

Figure 4: Relationships between the sunflower yield and its growth parameters (number of heads per ha as affected by (a) year and (b) fertilizer treatment and numbr of achnes per ha as affected by (c) year and (d) fertilizer treatment).

nibbling and lately by bird predation, together with a massive development of fungal diseases and other pathogens (*Sclerotinia sclerotiorum*) (2008), precipitation shortage (2009, 2012).

The other parameters, such as the weight of achenes per head, weight of thousand achenes, number of achenes per head and number of heads per ha were also influenced mainly by weather conditions, as they varied between years, but not between fertilization treatments. These results are in contrary to those of Ali *et al.* (2012), who with rising rates of N up to 150 kg ha^{-1} recorded an increasing weight of thousand achenes and positive effect of increasing rates of N on the number of achenes per head. Similar results published Salehi and Bahrani (2000).

It seems that high temperatures during a season decrease the weight of achenes per head, while lower temperatures increase that parameter. Also, the lowest number of achenes per head, accompanied with the highest weight of thousand achenes, the relatively high weight of achenes per head (second highest in the experiment) and with the lowest achenes yield and aboveground biomass of the plant, was recorded in 2008, the year with the lowest amount of precipitation (April–October 49.89 mm). The highest number of achenes per head was accompanied with the highest yield and weight of achenes per head in the year 2010. Correlation analysis revealed a positive relationship between the number of achenes per head and yield ($r = 0.38$).

Relatively poor response of sunflower to fertilizer treatment can be explained by a high initial content of soil nutrients (Tables 1 and 2). Our results show the possibility of the highly fertile soils in the Czech Republic lowlands to provide an excellent conditions for high sunflower yields. The average achene yield in the Czech Republic ranged from 2.1 to 2.5 t ha^{-1} between 2009 and 2012. At the same time, in France and Germany, European biggest sunflower producers, the average yields ranged from 3.3 to 3.8 and 2.9 to 4.3 t ha^{-1}, respectively (European Commission report 2013). A gap between actual and potential yield estimated in our experiment can be explained by longtime application of mineral and organic fertilizers and by strict adhering of crop cycling on the site.

Application of B and Zn provided 8%, respectively 9% achenes yield increase when compared to C treatment. Concerning the initial total content of B and Zn in the soil, it seems that the yield increase was achieved mainly by other factors, such as the N application and applied micronutrients played only supporting role. Differences between N 90 and N 90 treatments accompanied with micronutrients were not significant. Further, the initial content of B in soil ranged from 68.55 (2008) to 84.62 (2009) mg kg^{-1}, which is, according to Gupta (2006), an average content of most agricultural soils. The total Zn content ranged from 56.28 (2011) to 63.97 (2012) mg kg^{-1}, which is also an average and a suitable content (Storey, 2006). Several experiments focused on the effect of micronutrients on achenes yield were done worldwide. Oyinlola (2007) recorded a significant achenes yield increase, following application of B. But the total content of B at the experimental site ranged only from 0.09 to 0.17 mg kg^{-1}. Al-Amery *et al.* (2011) discovered a positive effect of application of B on the achenes yield, but the experiment was done on the soil with B content ranging only from 11 to 16 mg kg^{-1}. Siddiqui *et al.* (2009) and Kumar *et al.* (2010) also discovered a positive relation between B and Zn application and achenes yield, but the initial content of those micronutrients in the soil was unknown. The suitable content of B and Zn in the soil of our experiment could be explained by a parent rock type, climatic conditions and by longtime application of different combinations of organic and mineral fertilizers, accompanied with optimized crop rotation. Li *et al.* (2007) published that 16 years of application of organic fertilizers significantly increased DTPA (diethylenetriamine pentaacetic acid)-extractable Zn and Fe content. In other research, after 19 years of application of mineral and organic fertilizers incorporated with straw, the concentration of soil total Cu, Zn, Fe and Mn increased (Li *et al.*, 2010). These factors could explain why not significant response of sunflower on applied micronutrients was recorded in our case, but was recorded in other, newly established experiment (Zukalová *et al.*, 2009). Application of Mo has not increased yield, although the initial total content was considered as low (total Mo content ranged from 0.11 (2009) to 0.27 (2011) mg kg^{-1}). According to Škarpa *et al.* (2013), the optimal Mo soil content ranges from 0.4 to 1 mg kg^{-1}. Our results are in contrary to Škarpa *et al.* (2013), who revealed a positive response of foliar application of Mo on the sunflower yield.

Conclusions

Application of high doses of the mineral N in fertile soil with the good mineralization ability was not accompanied by a significant increase in achenes and biomass yield. On the contrary, the increase in N application rate above 60 kg N ha^{-1} was accompanied by a decrease in achenes yield. Application of 120, 90 and 60 kg N ha^{-1} provided an average yield 4.01, 4.14 and 4.34 t ha^{-1}, respectively. Micronutrients (B, Zn a Mo) were applied together with 90 kg N ha^{-1}. Application of B and Zn achieved slightly higher, while the addition of Mo slightly lower achenes yields when compared to the treatment with the same level of N and without micronutrients addition. Application of micronutrients at the sites with sufficient content of those micronutrients in soil had no statistically significant effect on achenes and biomass yield. Achenes and biomass yield was primarily affected by year to year fluctuations due to weather conditions which affected plant growth, disease and predatory occurrence. The weight of achenes per head, weight of thousand achenes and the number of achenes per head were not significantly affected by fertilizer treatment overall experiment, but significant differences were recorded in particular years, demonstrating high effect of weather conditions on individual yield components.

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