

CHANGES IN SELECTED PRODUCTION PARAMETERS AND FATTY ACID COMPOSITION OF SUNFLOWER (*HELIANTHUS ANNUUS*, L.) IN RESPONSE TO NITROGEN AND PHOSPHORUS APPLICATIONS

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Abstract

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The effect of N and P fertilization on the dry matter content, concentration of nutrients (N, P, K, Ca and Mg) and their intake by the plant, production characteristics (achene yields, head diameter, 1000-seed weight, oil content and oil production) and the content of fatty acids (palmitic, stearic, palmitoleic, oleic, linoleic and linolenic) was evaluated in the one-year pot trial with sunflower (*Helianthus annuus*, L.). Nitrogen application had a positive effect on dry matter production and increased N intake by plants as early as the beginning of vegetation (4th true leaf). The effect of P on dry matter production was not markedly evident until the later growth stage (10th true leaf). At the end of the vegetation period the weight of plants of variants fertilised with N only and variants where P was applied evened out. Achene yields, head diameters, 1000-seed weight, oil content and oil production increased statistically significantly after N application. Of all the monitored characteristics the increased level of P in the soil affected only the 1000-seed weight. The application of both nutrients did not significantly change the content of fatty acids.

N fertilization, P fertilization, achene's yields, head diameter, 1000-seed weight, oil content, fatty acids

Sunflower plays an important role in crop rotations in the Czech Republic. It is a traditional crop and has a high genetic potential with yields of as much as 5 tons per hectare, which ranks it among traditional oil crops. Although we see year-on-year fluctuations in the cultivation areas in the Czech Republic, in terms of sunflower they are gradually increasing. Also the increasing hectare yields are an important factor.

Sunflower is one of the main thermophile oil plants (LAGRAVÈRE et al., 2004; ZUBILLAGA et al., 2002) and its importance is growing in view of the climate development, both in the Czech Republic and, in particular, on a global scale. Growing sunflower is all the more justified because it is a convenient crop

and can be used as a substitute crop after ploughing back frost-killed winter crops on whole areas.

A number of agricultural measures must be carried out when growing sunflower; important is plant nutrition based on the rational use of fertilisers, which increase the soil supply of nutrients, at the same time avoiding any negative impacts on the sustainability of the agricultural production system. A good supply of nutrients in the soil is one of the basic prerequisites of economically effective yields of high quality. Nitrogen plays an important and irreplaceable role as a limiting factor influencing sunflower production (ÖZER et al., 2004; ZUBILLAGA et al., 2002). Nitrogen fertilisation contributes namely to the production of biomass, it increases achene yields and oil production (RUFFO et al., 2003). N is

an important nutrient participating in proteosynthesis (PASDA et al., 1991). It is an important component of chlorophyll and is involved in the metabolism of vitamins and other bio-catalytic substances. In terms of yields, phosphorus may be a limiting factor in some soils (ZUBILLAGA et al., 2002). To begin with phosphorus is a component of phospholipids, phosphorylated saccharides and peptides, nucleic acids, adenylylates (NAD^+ , NADP^+) and ADP and ATP compounds. Its part in metabolic processes is associated with signal transmission, use and conversion of energy. Both nutrients contribute to the metabolism of plant enzymes important in the biochemistry of lipid metabolism, which catalyse reactions from acetyl coenzyme A to the final products. In common with other vegetable oils approximately 95–99 % of the fatty acids in sunflower are present as triacylglycerols.

The objective of the pot trial was to explore the effect of N and P fertilisation on the intake of nutrients and their content in plant matter, on achene yields, head diameter, 1000-seed weight, oil content, oil production and quality of oil in terms of the content of fatty acids in sunflower.

MATERIALS AND METHODS

The one-year pot trial with sunflower (*Helianthus annuus*, L.) was established in the experimental vegetation hall of the Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition of the Faculty of Agronomy, Mendel University of Agriculture and Forestry in Brno in 2006.

Soil characteristics

In autumn 2005 we filled each pot with 11 kg of loamy soil characterised as cambisol (FAO soil taxonomy). One week before sowing the soil was sampled for analysis. Table I gives the basic soil characteristics. We monitored the following parameters: pH (1:5 DM soil:solution of 0.01M CaCl_2) and available nutrients (P, K, Ca and Mg) set down for soil extraction with the leaching agent Mehlich 3 (0.015 M NH_4F + 0.20 M CH_3COOH + 0.25 M NH_4NO_3 + 0.013 M HNO_3 + 0.0005 M EDTA) (1:10 DM soil:extraction agent Mehlich 3) (JONES, 1990). The content of phosphorus from the extract was determined by colorimeter (UV/VIS spectrophotometer, ATI Unicam 8625). Potassium, calcium and magnesium were assessed by means of atomic absorption spectrophotometer (AAS) on a Carl Zeiss Jena AAS-30 apparatus.

I: Agrochemical characteristics of the soil in mg.kg^{-1} DM soil

pH/ CaCl_2	Content of available nutrients in mg.kg^{-1} (Mehlich 3)			
	P	K	Ca	Mg
5.8	42.4	225.4	4283	409.7

Arrangement and treatment of the trial

The sunflower variety Jazzy (early hybrid giving high achene yields, high to very high oil content and resistance against European strains of sunflower rust) was sown on 31 May, two plants per pot, in four replications. After sowing, the fertilisers were applied in the form of a solution according to a pattern given in Table II. In variant 3 phosphorus was applied to a level of 81 mg.kg^{-1} (category of soil supply with available P according to Mehlich 3 – good level), in variant 4 to a level of 116 mg.kg^{-1} (category of soil supply with available P according to Mehlich 3 – high level) (TRÁVNÍK et al., 1999). The entire aboveground part of sunflower intended for chemical analysis was sampled in the stage of the 4th and

10th true leaf [20 June (20 days after sowing: DAS), 4 July (34 DAS), respectively]. In the stage of the 14th–16th true leaf [21 July (51 DAS)] sunflower of variants 2–4 was fertilised with 1g per pot of nitrogen (ammonium nitrate). Over the whole vegetation period the water content in the pots was maintained at 60% of the maximal water capacity. Due to a favorable climate of the year and low occurrence of harmful factors at the place of the experiment, in particular, it was not necessary to apply any plant protection means. Sunflower was harvested at physiological maturity on 3 October 2006 (125 DAS). For one week prior to harvest the plants were not watered and they dried up naturally.

II: Treatments of experiment

No. var.	Variant of fertilization	Doses of nutrients (g pot^{-1})	Fertilizers
1.	no fertilizer	0	0
2.	N	1g N pot^{-1}	ammonium nitrate
3.	N + P	1g N + 0.478 g P_2O_5 pot^{-1}	Amofos + ammonium nitrate
4.	N + 2P	1g N + 1.360 g P_2O_5 pot^{-1}	Amofos + ammonium nitrate

Ammonium nitrate (34 % N), Amofos (12 % N, 52 % P_2O_5)

Evaluated characteristics

The experiment was evaluated in the stage of the 4th and 10th true leaf, namely: the dry matter content, concentration of nutrients (N, P, K, Ca and Mg) and their intake by the plant; after harvest we evaluated: the dry matter content, concentration of nutrients (N, P, K, Ca and Mg), production characteristics (achene yields, head diameter, 1000-seed weight, oil content and oil production) and the content of fatty acids (palmitic, stearic, palmitoleic, oleic, linoleic and linolenic). Samples of the plant matter (in the stage of the 4th and 10th true leaf – the whole aboveground part of the plants; after harvest – the whole aboveground part of the plants deprived of achenes) were dried at 60 °C, ground in a grinder and homogenized. This plant matter was subjected to wet mineralization (H_2SO_4 a H_2O_2) (ZBÍRAL, 1994). Nitrogen concentration was assessed using the method according to Kjeldahl. The contents of potassium, calcium and magnesium were assessed using the atomic absorption spectrophotometer (AAS) on a Carl Zeiss Jena AAS-30 apparatus. Colorimetry was used to assess the content of phosphorus from the extract on a UV/VIS spectrophotometer, ATI Unicam 8625. The oil content was determined by extraction of sunflower achenes in a continuous flow extractor using Soxhlet's method. Fatty acid contents were determined as methyl esters using gas chromatography (HOUGEN and BODO, 1973).

Statistical evaluation

For the evaluations we used the programme Statistica 7.1 CZ. The effect of fertilisation on the monitored characteristics was evaluated by uni-factorial analysis of variance (ANOVA) according to the model equation: $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where y_{ij} is the effect variable; μ total mean; α_i deviation of the mean of the i -teenth group according to one sorting factor and ε_{ij} are random disturbance deviations. The dif-

ferences among the variants were assessed by tests according to Tukey at a 95% ($P < 0.05$), 99% ($P < 0.01$) and 99.9% ($P < 0.001$) level of significance.

RESULTS AND DISCUSSION

Nutrients in the plant matter

The positive effect of applications of nitrogenous fertilisers on the nitrogen content in the plant appeared as early as the stage of the 4th true leaf (Tab. III). Nitrogen concentration relatively increased in the nitrogen-fertilised variant (variant 2) by 13.0 % compared to the control (variant 1). LOUBSER and HUMAN (1993a) and LOPEZ-BELLIDO et al. (2002) drew similar conclusions. Nitrogen application also increased dry matter production (variant 2), which was positively reflected in intensive nitrogen uptake (Tab. IV). STEER et al. (1986) presented similar conclusions. LOUBSER and HUMAN (1993b) reported that N application also increased the concentration of P, but only in the initial stage of growth. This was not confirmed in our experiment. But not only nitrogen had a positive effect on dry matter production. Increasing the level of phosphorus to 116 mg.kg⁻¹ (variant 4), the dry matter of sunflower increased by 54.2% compared to the control variant. The dry matter production of this variant was also higher than the variant fertilised only with nitrogen (by 32.1 %). The application of P fertilisers increased the concentration of phosphorus in the plant matter (Tab. III) and consequently the total intake of phosphorus by the plant (Tab. IV). Likewise LOUBSER and HUMAN (1993b) reported an increased concentration of phosphorus after the application of P fertilisers virtually all through the vegetation period. A higher production of dry matter in the fertilised variants (2–4) reduced potassium and calcium concentrations in the plant matter.

III: Dry matter weights and nutrients concentration at the 4-true leaf (20.06.2006)

No. var.	Variant of fertilization	Dry matter weight (g per plant)	Nutrients concentration (% DM)				
			N	P	K	Ca	Mg
1.	no fertilizer	0.24	4.69	0.43	5.25	1.79	0.53
2.	N	0.28	5.30	0.39	5.18	1.50	0.53
3.	N + P	0.27	5.92	0.68	4.58	1.39	0.56
4.	N + 2P	0.37	5.54	0.81	4.63	1.46	0.62

IV: Nutrient uptake (mg per plant) at the 4-true leaf (20.06.2006)

No. var.	Variant of fertilization	N	P	K	Ca	Mg
1.	no fertilizer	11.36	1.05	12.71	4.33	1.28
2.	N	15.06	1.12	14.72	4.27	1.52
3.	N + P	15.97	1.83	12.36	3.74	1.52
4.	N + 2P	20.27	2.95	16.93	5.36	2.25

Fourteen days after analyzing the first sampling the second sample was taken, i.e. in the stage of the 10th true leaf, and was compared with values detected in the preceding analysis (Tab. III). In the stage of the 10th true leaf the difference in dry matter production between the nitrogen-fertilised variant and the control decreased (Tab. V) whereas nitrogen concentration in the plant matter in the 2nd variant more than doubled. HOCKING et al. (1987) also reported that an increased concentration of N after the application of N fertilisers was monitored primarily in the vegetative parts of the plants. The considera-

ble increase in dry matter production of the above-ground matter after the application of P fertilisers reduced the content of nitrogen and potassium in the plant matter (Tab. V). Analogous to the analysis in the stage of the 4th true leaf phosphorus contributed significantly to its higher concentration in the plant matter. Compared to the N-fertilised variant 2 the intake of nutrients (mg.plant⁻¹) after phosphorus fertilisation increased in variants 3 and 4; nitrogen increased by 61.3 % and 71.9 %, respectively, and phosphorus by 131.7 % and 188.0 %, respectively (Tab. VI).

V: Dry matter weights and nutrients concentration at the 10-true leaf (04.07.2006)

No. var.	Variant of fertilization	Dry matter weight (g per plant)	Nutrients concentration (% DM)				
			N	P	K	Ca	Mg
1.	no fertilizer	2.93	2.00	0.43	6.01	1.25	0.36
2.	N	3.04	4.03	0.38	6.17	1.79	0.74
3.	N + P	5.24	3.77	0.49	5.25	1.39	0.81
4.	N + 2P	5.70	3.69	0.59	5.10	1.62	0.77

VI: Nutrient uptake (mg per plant) at the 10-true leaf (04.07.2006)

No. var.	Variant of fertilization	N	P	K	Ca	Mg
1.	no fertilizer	58.63	12.64	175.81	36.69	10.42
2.	N	122.42	11.62	187.30	54.49	22.48
3.	N + P	197.43	25.92	274.88	72.77	42.32
4.	N + 2P	210.46	33.46	290.40	92.35	43.78

After harvesting the dry matter of sunflower deprived of achenes was weighed. The effect of N fertilisation on the dry matter weight was significant [$F(3;12) = 155.54$; $P < 0.001$]. The dry matter production of this N-fertilised variant more than doubled when compared to the control (variant 1) (Tab. VII). Likewise MAHAL et al. (1998) and ÖZER et al. (2004) reported a significant effect of graded doses of N fertilisers on the weight of plants after harvest. On top of that HOCKING et al. (1989) stated that the nitrogen deficit delayed all developmental stages of sunflower, including seed maturation, and that its deficit reduced plant weight, leaf area and number of

leaves. In our experiment the effect of phosphorus on the weight of the aboveground part of sunflower was not confirmed. The results show that the plant weight in variants where P fertilisers were applied decreased (variants 3 and 4) to 95.1 and 94.2 %, respectively, of the weight of variant 2. These conclusions are inconsistent with results of experiments with phosphorus as reported by RODRIGUEZ et al. (1998) who concluded that the reduction of the leaf area was due to the direct effect of the P deficit, which contributed namely to the reduction of the production of assimilates necessary for sunflower growth.

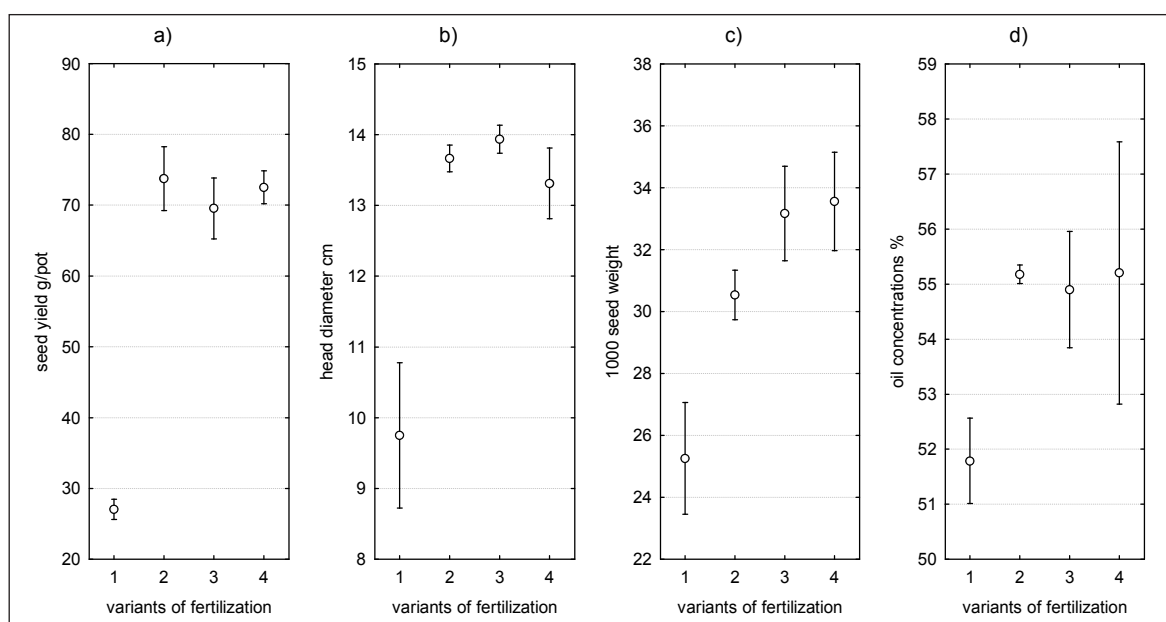
VII: Dry matter weights and nutrients concentration after harvest

No. var.	Variant of fertilization	Dry matter weight (g per plant)	Nutrients concentration (% DM)				
			N	P	K	Ca	Mg
1.	no fertilizer	25.00	0.43	0.39	3.68	1.70	0.26
2.	N	52.65	0.63	0.07	2.52	1.48	0.50
3.	N + P	50.06	0.61	0.07	2.09	1.38	0.52
4.	N + 2P	49.58	0.60	0.10	1.91	1.33	0.52

Achene yields, head diameter, 1000-seed weight, oil content and oil production

The application of graded doses of nutrients, particularly nitrogen, did not have a statistically significant effect on achene production [$F(3;12) = 445.22$; $P < 0.001$] and size of head [$F(3;12) = 112.86$; $P < 0.001$] (Fig. 1). The yields of the variant fertilised with nitrogen increased only 1.5 times compared to the control variant. SCHEINER and LAVADO (1999), TOMAR et al. (1999), RUFFO et al. (2003) and ÖZER et al. (2004) also observed that nitrogen had a positive effect on achene yields and head diameter. SCHEINER et al. (2002) discovered that after N application the production of sunflower achenes increased by 17 %. HOCKING and STEER (1989), BANGE et al. (1997), SCHEINER et al. (2002) and ZU-

BILLAGA et al. (2002) reported that a higher achene production after the application of N fertilisers was due to the higher number of achenes in the head. N application (variant 2) increased the head diameter by almost 40 %. A positive correlation was discovered between achene yields and size of head expressed by the correlation coefficient 0.964. Phosphorus fertilisation had no significant effect ($P < 0.05$) on achene production and size of inflorescence (Fig. 1). In both variants fertilised with this nutrient (variants 3 and 4) these two production characteristics ranged at levels comparable with the variant fertiliser with nitrogen only. SCHEINER and LAVADO (1999) and COLOMB et al. (2007) discovered that phosphorus had little to say in achene yields, while NAPHADE and NAPHADE (1991) reported that achene yields increased due to graded doses of P.



1: Effect of N and P fertilization on sunflower (a) seed yield, (b) head diameter; (c) 1000 seed weight and (d) oil concentrations. Errors bars represent Tukey at $P < 0.05$

Fertilisation also had a positive effect on the 1000-seed weight [$F(3;12) = 67.85$; $P < 0.001$]. Compared to the control, in the variant fertilised only with nitrogen the 1000-seed weight increased relatively by 20.9 %. STEER et al. (1986), HOCKING et al. (1987), MAHAL et al. (1998), TOMAR et al. (1999) and ÖZER et al. (2004) drew the same conclusion. On the contrary SCHEINER et al. (2002) discovered no change in achene weight due to N fertilisation. As Fig. 1 shows the only yield characteristic where the difference between the N fertilised variant 2 and variants where phosphorus was applied (3 and 4) was significant was the 1000-seed weight. The relative growth in weight of these variants was 8.6 and 9.9 %, respectively. TAMAK et al. (1997) and TOMAR et al. (1999) also reported increased seed weight due to increased doses of P fertilisers.

Against the findings of a number of authors (BLAMEY and CHAPMAN, 1981; BOZKURT and KARACAL, 2001; HOCKING et al., 1987; PASDA and DIEPENBROCK, 1991; RUFFO et al., 2003; SCHEINER et al., 2002. and STEER et al., 1986) the application of N fertilisers increased the oil content in sunflower seeds [$F(3;12) = 15.02$; $P < 0.001$]. The difference between the control variant and variant 2 was 6.6 % rel. ZUBILLAGA et al. (2002) presented similar conclusions; in 1998–1999 they discovered that the oil content increased after the application of nitrogen (from 53.10 to 53.46 %). The application of N fertilisers increased oil production (g.pot^{-1}) particularly because the yield of achenes was higher. In the control variant oil production reached 14.0 g.pot^{-1} , while in variant 2 it increased to 40.7 g.pot^{-1} . STEER et al. (1986), STEER and SEILER

(1990) and RUFFO et al. (2003) confirmed that N fertilisation increased oil production per unit of area. Fig. 1 shows that phosphorus application had no effect on the oil content and the values (variants 3 and 4) ranged at levels achieved by nitrogenous fertilisation. On the other hand BLAMEY and CHAPMAN (1981) and TAMAK et al. (1997) reported that phosphorus had a positive effect on the oil content and oil production per area.

Fatty acids

BOZKURT and KARACAL (2001) indicated a significant correlation between the concentration of fatty acids contained in sunflower oil and the content of nutrients in achenes. They attributed the highest correlation in relation to nutrients to oleic and linoleic acid. In our experiment the effect of nitrogen and phosphorus on the quality of oil in terms of the content of fatty acids was not significant ($P < 0.05$) (Tab. VIII). Under the effect of N fertilisation

the content of stearic acid [$F(3;12) = 0.54$; $P = 0.6647$], palmitoleic acid [$F(3;12) = 0.78$; $P = 0.5298$] and linoleic acid [$F(3;12) = 0.68$; $P = 0.5810$] decreased by 3.7, 9.5 and 5.9 %, respectively. N application had virtually no effect on the concentration of palmitic acid [$F(3;12) = 0.13$; $P = 0.9374$] and linoleic acid [$F(3;12) = 0.06$; $P = 0.9787$]. Only oleic acid [$F(3;12) = 1.04$; $P = 0.4104$] showed a positive response to N fertilisation and increased to 2.7 %. BOZKURT and KARACAL (2001) also discovered that N had a positive effect on the content of oleic acid. The experiments of STEER and SEILER (1990) likewise showed that nitrogen doses considerably affected the amount of palmitic, stearic, oleic and linoleic acid in sunflower oil. BAHL et al. (1997) pointed out that after the application of N fertilisers the ratio between oleic and linoleic acid, as an important indicator of the edible quality of the oil, increased. This fact was confirmed in our experiment when this ratio increased from 0.2068 (variant 1) to 0.2129 (variant 2).

VIII: The fatty acid composition of seed oil (values are percent of total fatty acid extracted from the seeds)

No. var.	Variant of fertilization	Palmitic 16:0	Stearic 18:0	Palmitoleic 16:1	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
1.	no fertilizer	6.35 <i>a</i>	3.71 <i>a</i>	0.070 <i>a</i>	15.38 <i>a</i>	74.36 <i>a</i>	0.128 <i>a</i>
2.	N	6.29 <i>a</i>	3.58 <i>a</i>	0.063 <i>a</i>	15.79 <i>a</i>	74.16 <i>a</i>	0.120 <i>a</i>
3.	N + P	6.35 <i>a</i>	3.61 <i>a</i>	0.063 <i>a</i>	15.70 <i>a</i>	74.15 <i>a</i>	0.130 <i>a</i>
4.	N + 2P	6.27 <i>a</i>	3.39 <i>a</i>	0.063 <i>a</i>	16.06 <i>a</i>	74.13 <i>a</i>	0.120 <i>a</i>

Values within columns followed by the same letter aren't significantly (Tukey, $P < 0.05$)

The effect of graded doses of phosphorus on the concentration of fatty acids was only sporadic. Compared to the N-fertilised variant 2, the effect of a lower dose of P (variant 3) was most evident in an increased content of linoleic acid (by 8.3 %). Contents of the other fatty acids differed only minimally (not more than ± 1 %). A higher dose of P (variant 4) increased the content of oleic acid only. A slight depression of the other acids was detected. BOZKURT and KARACAL (2001) reported that correlation between oleic acid and the phosphorus concentration was positive and was expressed by the correlation coefficient 0.558.

In our experiment we did not detect a significant effect of fertilisation on the content of fatty acids. Crucial for the oil quality is the time of sowing (GUPTA et al., 1994). Early sowing of sunflower increases the content of oleic acid; later sowing increases the content of linoleic acid. The oil quality is also considerably affected by the temperature during synthesis of lipids in the achenes (SEILER, 1986 and UNGER, 1980). High temperatures produce less linoleic acid and increase the proportion of oleic acid.

SUMMARY

Nitrogen application had a positive effect on dry matter production and increased N uptake by plants as early as the beginning of vegetation (4th true leaf). The effect of P on dry matter production was not markedly evident until the later growth stage (10th true leaf). At the end of the vegetation period the weight of plants of variants fertilised with N only and variants where P was applied evened out. Achene yields, head diameters, 1000-seed weight, oil content and oil production increased statistically significantly after N application. Of all the monitored characteristics the increased level of P in the soil affected only the 1000-seed weight. The application of both nutrients did not significantly change the content of fatty acids.

SOUHRN

Změny ve vybraných produkčních parametrech a ve složení mastných kyselin slunečnice (*Helianthus annuus*, L.) v závislosti na aplikaci dusíku a fosforu

V jednoletém nádobovém vegetačním pokusu byl sledován vliv N a P hnojení na obsah sušiny, koncentrace živin (N, P, K, Ca a Mg) a jejich odběr rostlinou, výnos nažek, průměr terče, hmotnost tisíce semen, olejnatost, produkce oleje a kvalitu oleje z pohledu zastoupení mastných kyselin (palmitová, stearová, palmitoolejová, olejová, linolová a linolenová) u slunečnice roční (*Helianthus annuus*, L.). Aplikace dusíku pozitivně ovlivnila produkci sušiny a zvýšila odběr N rostlinou již na počátku vegetace (4. pravý list). Fosfor se na produkci sušiny výrazně projevil až v pozdější fázi růstu (10. pravý list). Na konci vegetace se hmotnost rostlin mezi variantami hnojenými pouze dusíkem a variantami s aplikací fosforu vyrovnala. Výnos nažek, průměr terčů, hmotnost tisíce semen, olejnatost a produkce oleje se statisticky průkazně zvýšily vlivem aplikace dusíku. Zvýšenou zásobou fosforu v půdě byla ze sledovaných charakteristik signifikantně ovlivněna pouze hmotnost tisíce semen. Obsah mastných kyselin se vlivem aplikace obou živin průkazně nezměnil. Přesto bylo zaznamenáno po N hnojení zvýšení koncentrace kyseliny olejové (18:1) a pokles obsahu kyseliny linolové (18:2). V návaznosti na to došlo ke zvýšení jejich poměru (kys. olejová : kys. linolová). Fosfor se na koncentraci sledovaných mastných kyselin výrazněji neprojevil.

dusíkaté a fosforečné hnojení, výnos nažek, průměr terče, hmotnost tisíce nažek, olejnatost, mastné kyseliny

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