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The effect of nitrogen form and air temperature during foliar fertilization on gas exchange, the yield and nutritive value of spinach (*Spinacia oleracea* L.)

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ABSTRACT

A pot experiment conducted in a growth chamber studied the effectiveness of foliar fertilization of spinach (*Spinacia oleracea* L.), with different forms of nitrogen applied with the air temperatures: 5°C, 15°C and 25°C. Nitrogen was supplied three times in the form of 1% water solutions of $CO(NH_2)_2$, NH_4NO_3, NH_4HCO_3 and $Ca(NO_3)_2 \times 4\ H_2O$, with water as the control. The obtained results showed that nitrogen in the form of $Ca(NO_3)_2 \times 4\ H_2O$ – independent of the air temperature during solution application – is the least useful in foliar fertilization of spinach leaves, whereas the effectiveness of the other forms was similar, although the best effects were given by NH_4NO_3. Application of all forms of nitrogen at the air temperature of 25°C had the most positive effect on the course of gas exchange

(stomatal conductivity, photosynthesis, transpiration). Despite that, the highest leaf yield with the highest content of nitrogen, total chlorophyll (*a* + *b*), carotenoids and vitamin C, and the lowest content of nitrates, was given by plants with foliar application of all nitrogen forms at the temperature of 15°C.

INTRODUCTION

The technology of spinach cultivation, like that of other leafy vegetables with a short period of vegetation, requires an application of relatively high nitrogen doses. According to Czuba (1996), nitrogen fertilizers are applied much too early in relation to the requirements of cultivated plants for nitrogen. The result is excessive accumulation of nitrates and considerable losses of nitrogen as a consequence of washing off $N\text{-}NO_3$ inside the soil profile, or – which is much worse – $N\text{-}NO_3$ moving up to the surface waters. Sapek (1996) reports that washing off nitrogen in Poland is within the range of 1 kg to over 40 kg N ha^{-1} yearly. In order to lessen the burden of plant production to the environment, top-dressing should be applied much more frequently, as it provides the element in the period of intensive growth of the cultivated plant, and foliar fertilization should be applied more commonly.

The effectiveness of foliar fertilization of plants with the solutions of nitrogen salt depends on the applied nitrogen form and the environmental conditions around the plants, especially the air temperature. In research papers, urea is most frequently applied in foliar fertilization of plants with nitrogen (Yamada et al. 1965, Yu and Liu 1995, Terjo-Tellez et al. 2005, Dimitrov et al. 2006, Guvenc et al. 2006), while much fewer papers deal with the comparison of the effectiveness of $CO(NH_2)_2$ and inorganic forms of nitrogen. Rodney (1952) in his studies on apple trees, and Bowman and Paul (1992), in their studies on ryegrass, found that foliar fertilization with urea, ammonium nitrogen and nitrate nitrogen had no effect on the content of N in the leaves of the examined plants. On the other hand, however, Wittwer et al. (1967) found a higher rate of N absorption by the leaves treated with urea as compared to nitrates and ammonium, which was also confirmed by Furuya and Umemiya (2002). Scarce studies point to ambiguous effectiveness of ammonium and nitrate forms of nitrogen in foliar fertilization of plants. Bowman and Paul (1992) did not find any differences in the rate of the absorption of N applied on ryegrass leaves in the form of $N\text{-}NH_4$ and $N\text{-}NO_3$, whereas the studies by Komosa (1990) conducted on tomatoes point to a higher absorption of N from saltpeter fertilizers as compared to ammonium ones.

The rate of ion entrance on the aboveground parts of plants mainly depends on the thickness and configuration of the wax layer covering the skin of the leaves and other plant organs. This is first of all a species property of plants, but also – as follows from scarce studies – a property modified by the air temperature.

According to Reed and Tukey (1982), in conditions of high air temperature the elements of epicuticular waxes take a vertical configuration, which decreases the covering of the leaf surface and, as a consequence, increases the absorption of mineral elements, which is also confirmed by Stover and Greene (2005) in reference to growth regulators. Norris (1974), however, did not find any relation between the accumulation of waxes and the absorption of mineral elements.

Considering the ambiguous results of the studies conducted so far in this field, research was undertaken with the aim of determining the effect of the three basic nitrogen forms (NH_2, NH_4, NO_3) as well as form $NH_4 + NO_3$, applied in three clearly differentiated air temperatures, on the course of the processes of gas exchange, the yielding and the nutritive value of spinach leaves.

MATERIAL AND METHODS

The experiment was conducted in a growth chamber in the period between 19 March and 28 April (I repetition) and 30 May and 9 June (II repetition) in 2007. ‘Matador’ spinach plants grew in pots with the volume of 1.5 dm^3 filled with subsoil for the sowing and planting of vegetable plants. One litre of the subsoil contained (mg dm^{-3}) 130 N-NO_3, 66 P, 165 K, 915 Ca and 141 Mg; pH in H_2O was 5.5, with a salinity of 1.02 g NaCl. The experiment was carried out with fluorescent lighting with the stream density in the range of PAR about 200 μmol m^{-2} s^{-1}, the day’s length at 14 hours and the temperature at 18/15°C (day/night). Each experiment consisted of 60 pots, with 2 spinach plants growing in one pot. In the third week of vegetation, 5 experimental series were prepared (each of 12 pots), where each consisted of 3 sub-series (4 pots each) corresponding to the air temperature in the period of the nitrogen salt solution application. The experimental series were sprayed with 1% water solutions of the following nitrogen salts: $CO(NH_2)_2$, NH_4NO_3, NH_4HCO_3, $Ca(NO_3)_2 \times 4\ H_2O$. Application of the solutions was performed at the following air temperatures: 5°C, 15°C, 25°C. The plants remained in these temperatures for 48 hours, i.e. 24 hours before spraying and 24 hours after. Foliar fertilization of plants with the enumerated forms of nitrogen was repeated in the fourth and fifth weeks of vegetation. The solutions were applied with a manual sprayer until the state of full moistening of the accessible area of the leaf was achieved.

Twenty-four hours after the last spraying and the successive 24 hours of the plants remaining at the temperature of 18/15°C (day/night), measurements were performed of the net photosynthesis, transpiration and stomatal conductivity of the leaves for vapour. The measurements were made in eight repetitions on fully developed concentric leaves of spinach rosettes, using an ADC infrared analyser with a LCA-4 leaf chamber gas analyser. At the time of registration, the temperature in the leaf chamber was 25°C, and the PAR intensity was the same as during the plants' growth. At the same time, samples were taken from the analogous leaves to determine the content of total nitrogen, nitrates, chlorophyll, carotenoids and vitamin C in the leaves. The content of these compounds was determined in 4 repetitions by means of the following methods: N-total – according to Kjeldahl; nitrates – according to Cataldo et al. (1975); total chlorophyll ($a + b$) – according to Arnon (1949); carotenoids – according to Britton (1985); vitamin C – according to Pijanowski et al. (1973). The results present the arithmetic mean obtained in both experiments. The experiments were conducted according to the bifactor cross-classification model, where each plant grown in the pot was treated as the repetition. The data were analysed with the use of the multivarious analysis of variance. Significant differences were detected using t-Tukey's multiply confidence intervals at $p = 0.05$.

RESULTS AND DISCUSSION

The results included in Tables 1 and 2 point out that the form of foliar application of nitrogen salts had a significant effect on the gas exchange of the leaves. Plants treated with all the applied salts showed significantly higher transpiration in comparison to the control, which in the case of photosynthesis was not significant, whereas only the effect of calcium nitrate was insignificant, in the case of stomatal conductivity,. At the same time it can be seen (Table 2) that the applied forms of nitrogen – except nitrates [$Ca(NO_3)_2 \times 4\ H_2O$] – significantly increased the fresh weight yield connected both with a greater number of leaves formed in the leaf rosettes and a greater area of particular leaves (unpublished data). It can be supposed on this basis that foliar fertilization of plants with nitrogen increases the number and the size of stomata in the leaves, and, consequently, the stomatal conductivity of the leaves. On the other hand, there is a direct relationship between the stomatal conductivity of the leaves and the intensity of their transpiration and photosynthesis. This confirms the fact that CO_2 penetrates into the assimilative parenchyma via the same way as the vapours leave the leaves. The positive effect of foliar fertilization of apple trees with urea on the their process of photosynthesis was also stressed by Yu and Liu (1995), while Trejo-Tellez et al. (2005) observed an increased activity of Rubisco enzyme in spinach leaves under the influence of urea. On the other hand, other authors (Dimitrov et al. 2006, Guvenc et al. 2006) confirmed the positive effect of foliar fertilization of plants with nitrogen in the form of $CO(NH_2)_2$ on the yield of leafy vegetables, while there are no papers on the effect of other forms of nitrogen.

Table 1. Effect of nitrogen forms and air temperature on stomatal conductance and transpiration of spinach

Nitrogen form	Air temperature (°C)			Mean	Air temperature (°C)			Mean
	5	15	25		5	15	25	
	Conductance (mol H_2O m^{-2} s^{-1})				Transpiration (mmol H_2O m^{-2} s^{-1})			
Control (H_2O)	0.07	0.09	0.24	0.13	0.79	1.43	2.67	1.63
$CO(NH_2)_2$	0.13	0.12	0.29	0.18	1.94	2.70	3.02	2.55
NH_4NO_3	0.12	0.14	0.27	0.18	1.47	2.45	3.55	2.49
NH_4HCO_3	0.10	0.12	0.24	0.15	1.34	1.92	3.31	2.19
$Ca(NO_3)_2 \times 4\ H_2O$	0.10	0.11	0.14	0.12	1.44	2.61	2.95	2.33
Mean	0.10	0.12	0.24		1.40	2.22	3.10	
$LSD_{0.05}$ for N forms				0.04				0.52
$LSD_{0.05}$ for temp.				0.03				0.34
$LSD_{0.05}$ for N forms × temp.				0.08				n.s.

Table 2. Effect of nitrogen forms and air temperature on photosynthesis and yield of spinach

Nitrogen form	Air temperature (°C)			Mean	Air temperature (°C)			Mean
	5	15	25		5	15	25	
	Photosynthesis (μmol CO_2 m^{-2} s^{-1})				Mass of leaves (g $plant^{-1}$)			
Control (H_2O)	5.50	5.21	8.94	6.55	10.2	10.4	10.1	10.2
$CO(NH_2)_2$	7.28	8.82	10.07	8.72	15.8	19.7	13.9	16.5
NH_4NO_3	7.08	11.49	10.88	9.82	19.5	22.2	15.2	19.0
NH_4HCO_3	5.93	8.23	9.86	8.01	12.8	15.1	12.7	13.5
$Ca(NO_3)_2 \times 4\ H_2O$	3.04	2.86	4.25	3.38	8.1	8.7	6.3	7.7
Mean	5.77	7.32	8.80		13.3	15.2	11.6	
$LSD_{0.05}$ for N forms				0.68				2.62
$LSD_{0.05}$ for temp.				0.45				1.73
$LSD_{0.05}$ for N forms × temp.				1.46				n.s.

Table 3. Effect of nitrogen forms and air temperature on content of total N and nitrates in fresh mass of spinach

Nitrogen form	Air temperature (°C)			Mean	Air temperature (°C)			Mean
	5	15	25		5	15	25	
	Total N (%)				Nitrates (%)			
Control (H_2O)	0.41	0.51	0.43	0.45	0.14	0.11	0.07	0.11
$CO(NH_2)_2$	0.45	0.51	0.49	0.48	0.32	0.30	0.37	0.33
NH_4NO_3	0.45	0.50	0.48	0.48	0.33	0.26	0.31	0.30
NH_4HCO_3	0.40	0.50	0.49	0.46	0.17	0.15	0.10	0.14
$Ca(NO_3)_2 \times 4\ H_2O$	0.47	0.57	0.50	0.51	0.28	0.21	0.28	0.26
Mean	0.44	0.52	0.48		0.25	0.21	0.23	
$LSD_{0.05}$ for N forms				0.05				0.04
$LSD_{0.05}$ for temp.				0.03				0.03
$LSD_{0.05}$ for N forms × temp.				n.s.				0.09

Table 4. Effect of nitrogen forms and air temperature on content of chlorophyll and corotenoids in spinach

Nitrogen form	Air temperature (°C)			Mean	Air temperature (°C)			Mean
	5	15	25		5	15	25	
	Chlorophyll (mg g^{-1} f.m.)				Carotenoids (mg g^{-1} f.m.)			
Control (H_2O)	1.42	1.51	1.77	1.57	2.40	2.32	2.50	2.41
$CO(NH_2)_2$	3.44	3.61	2.84	3.30	3.94	4.36	4.48	4.26
NH_4NO_3	3.71	4.07	3.38	3.72	3.96	5.09	4.47	4.51
NH_4HCO_3	3.24	3.13	3.22	3.20	3.71	4.95	4.11	4.26
$Ca(NO_3)_2 \times 4\ H_2O$	1.86	2.19	2.32	2.12	2.32	2.98	2.73	2.68
Mean	2.73	2.90	2.71		3.27	3.94	3.66	
$LSD_{0.05}$ for N forms				0.36				0.41
$LSD_{0.05}$ for temp.				n.s.				0.27
$LSD_{0.05}$ for N forms × temp.				0.80				0.89

The results obtained in the paper also point out that the air temperature significantly determined the effectiveness of foliar application of different forms of nitrogen in the period of the treatment. The lowest stomatal conductivity, transpiration and photosynthesis were found at the temperature of 5°C. An increase of the temperature to 15°C – and even more to 25°C – significantly increased the value of the analyzed parameters of gas exchange. It is clearly seen that was related to the effect of air temperature on the total metabolic activity of plant leaves, as an analogous reaction was also shown by the leaves of control plants; however, in the case of stomatal conductivity and photosynthesis, the studies discovered an interaction of temperature and the form of nitrogen (Tables 1 and 2).

The effect of the air temperatures on the obtained weight of spinach leaves was different since the highest yield was given by plants with foliar application of nitrogen at the temperature of 15°C, while the lowest was observed at the temperature of 25°C. This points out that spinach is not a stenothermal plant and although photosynthesis in the periods of nitrogen solution application progressed more intensively in higher temperatures, this had no effect on the plants' growth in the periods between the spraying, when the temperature was 18/15°C.

Results included in Table 3 point out that the foliar application of nitrogen forms showed only a slight effect on the content of N-total in spinach leaves. Only foliar treatment of plants with $Ca(NO_3)_2 \times 4\ H_2O$ had a significant effect on the increase of nitrogen content. The researchers' opinions on this subject differ. Wittwer et al. (1967) and Furuya and Umemiya (2002) found a higher rate of N absorption by plants treated with urea as compared to nitrates and ammonium, whereas Bowmann and Paul (1992) did not observe any differences in this respect in plants treated with urea, ammonium and nitrate nitrogen, and Komosa (1990) found a higher absorption of N from fertilizers containing $N\text{-}NO_3$ in comparison to $N\text{-}NH_4$. The effect of the applied nitrogen forms on the content of nitrates in the leaves was much clearer since – independently of the temperature in the period of solution application – the fertilization of plants with urea, ammonium nitrate and calcium nitrate increased the percentage content of NO_3^- in spinach leaves three times, on average, and only the effect of ammonium carbonate was insignificant. These data in reference to the salts containing NO_3^- [NH_4NO_3, $Ca(NO_3)_2$] ions seem obvious, while being incomprehensible in relation to $CO(NH_2)_2$. The effect of air temperature on the percentage content of N-total and NO_3 in the leaves of the examined plants was completely different. The most total nitrogen was accumulated by plants from the solutions applied at the temperature of 15°C, whereas the most nitrates were accumulated at the temperature of 5°C, and the least at the temperature of 15°C. Hence, it seems that in the case of spinach the absorption of nitrogen applied onto the leaves proceeded for a longer period than 24 hours after the spraying and it mainly took place at the standard temperature of plants' growth, 18/15°C. These data also suggest – which is in accordance with other authors (Norris 1974, Reed and Tukey 1982) – that in the conditions of intensive increase of the leaf area (Table 2), the layer of epicuticular waxes covering the leaves becomes thinner, which increases the absorption of N supplied onto the leaf. It is also possible that the best metabolism of the N-NO_3 form to N-total is at the temperature of 15°C.

All nitrogen forms supplied by foliar application showed a highly significant effect on the content of chlorophyll and carotenoids in spinach leaves, with an exception of the slight influence of $Ca(NO_3)_2 \times 4\ H_2O$ on the content of carotenoids. The studies by Trejo-Tellez et al. (2005) of spinach and the studies by Yu and Liu (1995) of apple trees confirm the thesis that foliar fertilization of plants with $CO(NH_2)_2$ increases the content of chlorophyll in the leaves, while there are no data on the effect of other nitrogen forms. The temperature in the period of solution application did not affect the content of chlorophyll in the leaves, but it did affect the content of carotenoids, the concentration of which was the highest at the air temperature of 15°C (Table 4). It seems that this is connected with the effect of the analysed factors on nitrogen absorption, which favours chlorophyll synthesis, as it is one of the latter's elements.

Foliar fertilization of plants with the applied forms of nitrogen had a completely different effect on the content of vitamin C in the leaves as compared to the earlier analysed properties of plants. The application of all nitrogen forms, except $Ca(NO_3)_2 \times 4\ H_2O$, introduced a significant drop of the content of vitamin C in comparison to the control, and the application of nitrate nitrogen significantly increased its content. Plants with foliar application of all N forms at the air temperature of 15°C (Table 5) contained the most vitamin C. It is hard to take an attitude to these data because the literature lacks any reports on this subject.

Table 5. Effect of nitrogen forms and air temperature on content of vitamin C in spinach (mg 100 g^{-1} f.m.)

Nitrogen form	Air temperature (°C)			Mean
	5	15	25	
Control (H_2O)	65.2	68.2	65.6	66.3
$CO(NH_2)_2$	55.5	63.1	63.9	60.8
NH_4NO_3	52.9	60.6	57.2	56.9
NH_4HCO_3	49.5	68.2	53.8	57.2
$Ca(NO_3)_2 \times 4\ H_2O$	60.6	78.8	75.0	71.5
Mean	56.7	67.8	63.1	
$LSD_{0.05}$ for N forms	1.14			
$LSD_{0.05}$ for temp.	0.74			
$LSD_{0.05}$ for N forms × temp.	2.54			

CONCLUSIONS

1. Nitrogen in the form $Ca(NO_3)_2 \times 4\ H_2O$ proved useful in the foliar fertilization of spinach leaves, while the effectiveness of 1% water solutions of $CO(NH_2)_2$, NH_4NO_3 and NH_4HCO_3 was similar, however, NH_4NO_3 was the most effective.
2. Application of all forms of nitrogen at the air temperature of 25°C had the most positive effect on gas exchange (stomatal conductivity, photosynthesis, transpiration). Despite that, the highest leaf yield with the highest content of nitrogen, chlorophyll, carotenoids and vitamin C, and the lowest content of nitrates, was found in plants with foliar application at the temperature of 15°C, irrespective of the nitrogen form.

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WPŁYW FORMY AZOTU I TEMPERATURY POWIETRZA W NAWOŻENIU DOLISTNYM NA WYMIANĘ GAZOWĄ, PLON I WARTOŚĆ ODŻYWCZĄ SZPINAKU (*SPINACIA OLERACEA* L.)

Streszczenie: W doświadczeniu wazonowym prowadzonym w fitotronie badano efektywność nawożenia dolistnego szpinaku (*Spinacia oleracea* L.) różnymi formami azotu stosowanymi przy trzech temperaturach powietrza: 5, 15 i 25°C. Azot podano 3-krotnie w formie 1% roztworów wodnych $CO(NH_2)_2$, NH_4NO_3, NH_4HCO_3 i $Ca(NO_3)_2 \times 4\ H_2O$ wobec wody jako kontroli. Uzyskane wyniki wykazały, że azot w formie $Ca(NO_3)_2 \times 4\ H_2O$ niezależnie od temperatury powietrza w okresie aplikacji roztworu jest najmniej przydatny w dolistnym nawożeniu roślin szpinaku, efektywność natomiast pozostałych form była zbliżona, przy czym najlepszy efekt uzyskano przy zastosowaniu NH_4NO_3. Najkorzystniej na przebieg wymiany gazowej w liściach (przewodnictwo szparkowe, fotosyntezę, transpirację) wpływało zastosowanie wszystkich form azotu przy temperaturze powietrza 25°C. Jednak najwyższy plon liści o największej zawartości azotu ogólnego, chlorofilu, karotenoidów, witaminy C, a najmniejszej azotanów wydały rośliny traktowane formami azotu przy temperaturze 15°C.

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