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The effect of placement and light conditions during foliar application of Insol U fertilizer on gas exchange, yield and the quality of spinach (*Spinacia oleracea* L.)

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ABSTRACT

Pot experiments conducted in a growth chamber studied the effectiveness of foliar fertilization of spinach (*Spinacia oleracea* L.) with macro- and microelements in the form of a 1% water solution of Insol U fertilizer applied with fluorescent light of the PAR intensity of 200 µmol m^{-2} s^{-1} and with dispersed daylight of the intensity of 600 µmol m^{-2} s^{-1}. Insol U was applied three times, supplied on the upper, lower or both sides of the leaf blades. The control consisted of plants sprayed with distilled water. The obtained results pointed out that foliar fertilization of spinach with Insol U significantly increased the leaf gas exchange, the yield of the fresh leaf mass, the content of vitamin C, chlorophyll, carotenoids, as well as potassium and phosphorus in the leaves. The application of the fertilizer onto the upper and the lower side of the leaf blade had the most positive effect on

the analysed property of the leaves. Independently of the side leaf, the application of the fertilizer at a higher light intensity had a significantly better consecutive effect on all the analysed properties of the leaves.

INTRODUCTION

Until recently a view was held that plant production in Poland and the related application of mineral fertilizers did not pose a threat to the environment. Thorough research on this problem pointed out, however, that the situation is different (Pondel 1989). This especially refers to plants of relatively high nutrition requirements and at the same time with a short period of vegetation. One hectare of spinach cultivation needs the application of about 80-90 kg N, 40-60 kg P_2O_5 and 80-120 kg K_2O (Orłowski 2000). Before sowing or planting, nitrogen is usually applied in a half-dose, while potassium and phosphorus are mostly supplied in whole doses. If a given element is poorly absorbed in the soil, which is the case with NO_3^- and PO_4^{-3} ions, there is a high probability of them being washed away inside the soil profile or to the surface water.

A solution to this problem, especially in intensive production of vegetables, may be a more common application of foliar fertilizers containing many elements, and also usually including microelements in the form of chelates necessary for the plants. The studies conducted so far point out that in the majority of cases foliar application of multi-element fertilizers increased the accumulation of fresh weight in the leaves, the plants' yielding, the chlorophyll content as well as N, P, K, Ca and Mn in the leaves (Sadd et al. 1990, Kołota and Osińska 2001, Ling and Silberbush 2002, Al-Qurashi 2005). However, studies can be found in the literature where foliar fertilization did not show any effect on the stomatal conductivity of the leaves, transpiration, photosynthesis or the content of N, P, K, Ca and Mg in leaves (Haq and Mallarino 2000, Nava-Sanches et al. 2004).

Undoubtedly, the effects of foliar fertilization depend on the plant species as well as the manner of application and the conditions during the period of solution application on plant leaves, especially the intensity of light. It is generally considered that light is conducive to the absorption of mineral elements by leaves (Jyung et al. 1965, Rains 1967, Shim et al. 1972). On the other hand, however, a number of researchers (Tribe et al. 1968, Hallam 1970, Macej 1970, Leece 1978) pointed out that intensive light favours the sedimentation of surface waxes and the appearance of a thicker cuticular membrane on the leaf area, which makes the solution penetration and the absorption of the elements by the leaves difficult. Finally, some authors did not find any relationship between the light intensity and the thickness of the cuticular membrane on the plant leaves (Reed and Tukey 1982, Darnell and Ferree 1983).

It is well documented in the literature that the bottom part of the leaf absorbs mineral elements more intensively than the upper one (Hull 1970, Schlegel and Schonherr 2002). Hull (1970) justifies that this results from a greater number of stomata in the leaves on the bottom side and from the thinner cuticular layer covering the leaf. The leaves of the examined cultivar of spinach had nearly three times as many stomata on the upper side. Hence, in the light of ambiguous data concerning the effect of light intensity on the absorption of mineral elements by the leaves as well as the distribution of stomatal apparatuses, which is different from that of the majority of dicotyledonous plants, it is interesting to determine the effect of those factors on the effectiveness of foliar fertilization of spinach plants with Insol U fertilizer.

MATERIAL AND METHODS

The experiment was conducted in a growth chamber in two periods: first, between 5 March and 15 April, and second, between 21 May and 29 June in 2007. The leaves of 'Matador' spinach grew for the period of six weeks in a growth chamber with fluorescent light intensity in the range of PAR 200 µmol m^{-2} s^{-1}, a photoperiod of 14/10 h (day/night) and an air temperature of 18/15°C. In one pot with a volume of 1.5 dm^3 and filled with clean sand, two plants grew, which were watered with distilled water, keeping the moisture at a level of 80% of field water capacity. After the plant emergence, ½ of the concentration of Hoagland medium was supplied, and a successive dose was provided in the same quantity a week later. Each experiment consisted of 40 pots, which were divided into four experimental combinations, differentiated in respect of the place of Insol U fertilizer application on the leaves: upper epidermis, lower epidermis, as well as both upper and lower epidermis. The fertilizer containing 10.5% $N\text{-}NH_2$, 1.5% $N\text{-}NO_3$, 4% P_2O_5, 6% K_2O and microelements was supplied onto the leaves in the form of a 1% water solution. The leaves of the control plants were at the same time sprayed on both sides of their blades with distilled water. Half of the plants from each experimental combination (five pots) had fertilizer applied with fluorescent light of the intensity of 200 µmol m^{-2} s^{-1}, whereas the other half received the fertilizer at dispersed daylight with the intensity of about 600 µmol m^{-2} s^{-1}, with similar temperature and air moisture. Foliar fertilization of plants with Insol U was carried out three times with weekly intervals. The solutions was introduced by means of a small manual sprayer, each time until a state of full moistening of the accessible area of the leaves was achieved. After the treatment, plants treated with Insol U in daylight remained in the same conditions for a further 10 hours, after which they were placed in a growth chamber.

Forty-eight hours after the last spraying, measurements were made of the stomatal conductivity in the leaves, transpiration and photosynthesis. The measurements were made in 10 plants on fully developed middle leaves of spinach rosettes using an ADC infrared analyser with leaf chamber LCA-4. The temperature in the leaf chamber during the measurements was 25°C, and PAR was 200 μmol m^{-2} s^{-1}. At the same time the samples of leaves were collected for crude protein, vitamin C, chlorophyll and carotenoid content determination. The content of the enumerated compounds was determined in four repetitions, for four randomly selected plants by means of the following methods: crude protein – according to Kjeldahl; vitamin C – according to Pijanowski et al. (1973), chlorophyll – according to Arnon (1949) and carotenoids – according to Britton (1985). Next, the plants were cut, and the fresh weight of particular plants and – after drying – the dry weight of particular plants was determined. In dry mass of leaves, after their mineralisation the content of K, Ca, P and Mg was determined. Leaf ash was dissolved and the ASA method was used to establish the content of potassium and calcium in the solution, whereas the colorimetric method was used to determine the content of phosphorus and magnesium, respectively with the use of vanado-molybdenum or titan yellow. The results present the arithmetic mean obtained in both experiments. The experiments were conducted according to a bifactor cross classifacation 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

Results, included in Table 1, point out that the application of Insol U fertilizer had a highly significant effect in comparison to the control on stomatal conductivity in spinach leaves. Stomatal apparatuses in the leaves treated with a solution of macro- and microelements were about twice as permeable for gas exchange than they were in the case of plants treated only with water. It was also found that the spraying of the upper or lower part of the leaves was significantly less advantageous than the simultaneous spraying of both leaf sides with the solution. It seems that this can be related to the basic mechanism of the movement of stomatal apparatuses, which is based on the changes of the osmotic potential. The application of the Insol U solution directly onto the leaf area caused an increase of the potential in stomatal cells as a result of the cells absorbing mineral elements. This, in turn, caused a greater uptake of water by those cells and an increase of the turgor in them, resulting in a higher degree of opening of the stomata. The application of Insol U, independently of the sprayed side of the leaves, had a similar effect on the intensity of transpiration and photosynthesis in spinach leaves, which seems completely clear, because the more open stomata are at the same time “broader gates” for the diffusion of water vapour from the inside of the leaves and CO_2 from the outside (Tables 1 and 2).

Table 1. Effect of place application of Insol U and light intensity (μmol m^{-2} s^{-1}) on stomatal conductance and transpiration of spinach

Place of application	Light intensity		Mean	Light intensity		Mean
	200	600		200	600	
	Conductance (mol H_2O m^{-2} s^{-1})			Transpiration (mmol H_2O m^{-2} s^{-1})		
Control (H_2O)	0.15	0.14	0.14	2.29	1.52	1.90
Upper epidermis	0.25	0.26	0.25	2.42	3.67	3.04
Lower epidermis	0.25	0.29	0.27	3.13	3.50	3.31
Both epidermis	0.30	0.36	0.33	4.02	4.50	4.26
Mean	0.24	0.26		2.96	3.30	
$LSD_{0.05}$ for place		0.03			0.29	
$LSD_{0.05}$ for light		n.s.			0.16	
$LSD_{0.05}$ for place × light		0.05			0.49	

Table 2. Effect of place application of Insol U and light intensity (μmol m^{-2} s^{-1}) on photosynthesis and yield of spinach

Place of application	Light intensity		Mean	Light intensity		Mean
	200	600		200	600	
	Photosynthesis (μmol CO_2 m^{-2} s^{-1})			Mass of leaves (g $plant^{-1}$)		
Control (H_2O)	5.74	5.93	5.83	10.0	10.3	10.1
Upper epidermis	7.05	7.65	7.35	12.4	13.8	13.1
Lower epidermis	7.00	8.87	7.93	13.1	15.7	14.4
Both epidermis	10.05	10.97	10.51	15.2	16.6	15.9
Mean	7.46	8.35		12.7	14.1	
$LSD_{0.05}$ for place		0.26			2.60	
$LSD_{0.05}$ for light		0.15			1.39	
$LSD_{0.05}$ for place × light		0.44			n.s.	

At the same time, it was found out that the course of the process of photosynthesis in those conditions had a very close relation to the yield of spinach leaves. The lowest yield was given by the control plants, whereas yield was far higher by the plants treated with Insol U, and within the plants with foliar fertilization significant differences in the height of the yield of leaves occurred only between the plants receiving the fertilizer solution onto the upper area of the leaves and onto both areas simultaneously (Table 2). The relationship between photosynthesis intensity and the yield of the leaves seems obvious. It is more difficult to explain the significance of differences concerning the yield between the plants receiving Insol U on the upper epidermis and the plants receiving the solution of the fertilizer on both sides of the leaves, with no such differences observed in case of treating only the lower epidermis with the solution of mineral elements. It seems that this can result from the rate of penetration of the elements supplied to the epidermis of particular sides of the leaves, which points out that mineral elements supplied to the upper epidermis are absorbed the worst relatively, while those applied to the lower epidermis are absorbed much better and those applied onto both leaf sides at the same time were absorbed the best. Such an interpretation finds confirmation in the studies by Hull (1970) and Schlegel and Schonherr (2002), who claim that the upper epidermis of the leaves is covered with a thicker cuticle with a greater number of waxes than the lower one, which makes the ion penetration much more difficult. The literature lacks any data concerning the effect of the application of foliar fertilizers on the upper or the lower sides of the leaves and the yielding. On the other hand, the obtained results confirmed the studies according to which foliar fertilization with the solution of N, P, and K increased the fresh weight of the leaves (Sadd et al. 1990, Kołota and Osińska 2001, Ling and Silberbush 2002, Al-Qurashi 2005).

A much smaller influence on the analysed properties of spinach leaves was exerted by the application of Insol U with different light intensities. Results included in Tables 1 and 2 point out that the applied ranges of the light had no significant effect on the stomatal conductivity of the leaves, while affecting transpiration and photosynthesis as well as the leaf yield from a plant. Probably, the higher values of the analyzed properties with the light of 600 µmol m^{-2} s^{-1} should be explained by a better absorption of nitrogen in those conditions, which is pointed at by the content of protein in the leaves as well as the content of other analysed macroelements (K, Mg, P) (Tables 3, 5 and 6).

Table 3. Effect of place application of Insol U and light intensity (µmol m^{-2} s^{-1}) on content of crude protein and vitamin C in spinach

Place of application	Light intensity		Mean	Light intensity		Mean
	200	600		200	600	
	Protein (mg g^{-1} f.m.)			Vitamin C (mg 100 g^{-1} f.m.)		
Control (H_2O)	26.0	31.8	28.9	87.4	104.1	95.7
Upper epidermis	28.0	32.3	30.1	106.9	135.8	121.3
Lower epidermis	28.2	32.1	30.1	110.8	136.7	123.7
Both epidermis	30.3	32.0	31.1	109.1	132.0	120.5
Mean	28.1	32.0		103.5	127.1	
$LSD_{0.05}$ for place		n.s.			1.16	
$LSD_{0.05}$ for light		2.16			0.59	
$LSD_{0.05}$ for place × light		n.s.			2.03	

Results included in Tables 3 and 4 point out that foliar fertilization of spinach with Insol U had no significant effect on the content of protein in the leaves, while significantly and positively affecting the content of vitamin C, chlorophyll and carotenoids. Saad et al. (1990) also found that foliar fertilization with an NPK solution had a positive effect on the content of chlorophyll and carotenoids in wheat leaves, while Ling and Silberbush (2002) observed a positive effect on the content of chlorophyll in maize leaves. The effect of the place of fertilizer application on the value of the analysed properties differed; the best influence on the content of vitamin C was exerted by the fertilizer supplied only on the lower epidermis, while on the content of chlorophyll by the fertilizer applied on the lower epidermis and on the lower and upper epidermis. In the case of carotenoids the effect was the best with the fertilizer applied on both leaf parts at the same time. The effect of light intensity in the period of fertilizer application was much more distinct. Independently of the place of Insol U application on the leaves, much more crude protein, vitamin C, chlorophyll and carotenoids were contained in the leaves where the fertilizer was supplied with higher light intensity (600 µmol m^{-2} s^{-1}). It seems that this should be linked with the consecutive effect of Insol U supplied in these conditions on a more intensive progress of photosynthesis and greater absorption of macroelements in the leaves (Tables 2, 5 and 6).

Table 4. Effect of place application of Insol U and light intensity (µmol m^{-2} s^{-1}) on content of chlorophyll and carotenoids in spinach

Place of application	Light intensity		Mean	Light intensity		Mean
	200	600		200	600	
	Chlorophyll (mg g^{-1} f.m.)			Carotenoids (mg g^{-1} f.m.)		
Control (H_2O)	2.09	2.06	2.07	2.81	3.68	3.24
Upper epidermis	2.30	2.63	2.46	2.92	3.78	3.35
Lower epidermis	2.34	2.73	2.53	3.24	3.85	3.54
Both epidermis	3.46	3.69	3.57	3.89	3.97	3.93
Mean	2.55	2.78		3.21	3.82	
$LSD_{0.05}$ for place		0.33			0.24	
$LSD_{0.05}$ for light		0.17			0.13	
$LSD_{0.05}$ for place × light		n.s.			n.s.	

Table 5. Effect of place application of Insol U and light intensity (µmol m^{-2} s^{-1}) on content of potassium and calcium in dry mass of spinach

Place of application	Light intensity		Mean	Light intensity		Mean
	200	600		200	600	
	Potassium (%)			Calcium (%)		
Control (H_2O)	4.28	4.35	4.31	2.20	2.16	2.18
Upper epidermis	4.63	4.79	4.71	1.98	2.03	2.00
Lower epidermis	4.89	4.98	4.93	2.00	2.10	2.05
Both epidermis	5.24	5.77	5.50	1.98	2.02	2.00
Mean	4.76	4.97		2.04	2.08	
$LSD_{0.05}$ for place		0.23			n.s.	
$LSD_{0.05}$ for light		0.11			n.s.	
$LSD_{0.05}$ for place × light		0.39			n.s.	

Table 6. Effect of place application of Insol U and light intensity (μmol m^{-2} s^{-1}) on content of phosphorus and magnesium in dry mass of spinach

Place of application	Light intensity		Mean	Light intensity		Mean
	200	600		200	600	
	Phosphorus (%)			Magnesium (%)		
Control (H_2O)	0.26	0.36	0.31	0.36	0.43	0.39
Upper epidermis	0.30	0.42	0.36	0.33	0.38	0.35
Lower epidermis	0.32	0.41	0.36	0.33	0.41	0.37
Both epidermis	0.37	0.44	0.40	0.36	0.40	0.38
Mean	0.31	0.41		0.34	0.40	
$LSD_{0.05}$ for place		0.03			0.03	
$LSD_{0.05}$ for light		0.02			0.02	
$LSD_{0.05}$ for place × light		0.05			n.s	

Foliar fertilization of spinach with Insol U also caused a significant increase of the content of potassium and phosphorus in the leaves, and a decrease of the content of magnesium as compared to the control. That was related to the content of the fertilizer (part Material and methods), which contained 6% K_2O and 4% P_2O_5 and had no calcium or magnesium; hence, their concentration with much greater leaf mass in the series with foliar fertilization decreased slightly. Al-Quarshi (2005) and Ling and Silberbush (2002) showed that foliar NPK fertilization had an effect on the increased content of those elements in the leaves of guava and maize, while no such dependence was observed in onions by Nava-Sanchez et al. (2004), and Haq and Mallarino (2000) found it only rarely in soybeans. It was also found in plants treated with the fertilizer solution that the most potassium, phosphorus and magnesium was contained in the leaves sprayed with Insol U on both sides as compared with the application on the upper or the lower side, which certainly results from a greater area where absorption takes place. On the other hand, no significant differences were observed in the content of K, Ca, P and Mg in the leaves in the cases where fertilizer was applied only on the upper or the lower epidermis of the leaf, although Hull (1970) and Schlegel and Schonherr (2002) found that the lower part of the leaves absorbed mineral elements better than the upper one. This might have resulted from the fact that spinach leaves contained almost three times more stomata in the upper epidermis as compared to the lower epidermis (unpublished data). The effect of light intensity in the period of fertilizer application on the plant was much more distinct. Independently of the side of the leaf, foliar fertilization with a higher light intensity affected a significantly higher content of K, P and Mg in the leaves. These data confirmed the previous studies by Jyung et al. (1965), Rains (1967) and Shim et al. (1972), while Reed and Tukey (1982) and Darnell and Ferree (1983) observed no such relationship.

CONCLUSIONS

1. Foliar fertilization of spinach with a solution of macro- and microelements (Insol U fertilizer) significantly increased the process of gas exchange in leaves, the yield, the content of vitamin C, chlorophyll and carotenoids as well as potassium and phosphorus. The application of the fertilizer onto the upper and the lower side of the leaf had the most positive effect on the analysed properties.
2. Fertilizer application with a higher light intensity (600 µmol m^{-2} s^{-1}) in relation to light with a lower intensity (200 µmol m^{-2} s^{-1}) had a significantly more positive effect on all the analysed properties of plants.
3. The application of foliar fertilizers containing macro- and microelements in the cultivation of leafy vegetables made it possible to obtain high and good quality yields with little use of mineral soil fertilizers.

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WPŁYW MIEJSCA I WARUNKÓW ŚWIETLNYCH PODCZAS DOLISTNEGO STOSOWANIA NAWOZU „INSOL U” NA WYMIANĘ GAZOWĄ, PLON I JAKOŚĆ SZPINAKU (*SPINACIA OLERACEA* L.)

Streszczenie: W doświadczeniach wazonowych prowadzonych w fitotronie badano efektywność nawożenia dolistnego szpinaku (*Spinacia oleracea* L.) makro- i mikroelementami w postaci 1% roztworu wodnego „Insol U” aplikowanego przy świetle fluorescencyjnym o intensywności w zakresie PAR 200 µmoli m^{-2} s^{-1} i świetle dziennym rozproszonym o intensywności 600 µmoli m^{-2} s^{-1}. „Insol U” zastosowano 3-krotnie podając go na górną, dolną lub obie strony blaszek liściowych. Kontrolę stanowiły rośliny opryskane wodą destylowaną. Uzyskane wyniki wykazały, że nawożenie dolistne szpinaku „Insolem U” istotnie zwiększyło przebieg wymiany gazowej w liściach roślin, plon oraz zawartość witaminy C, chlorofilu, karotenoidów, a także potasu i fosforu. Najkorzystniej na analizowane parametry liści wpływała jednoczesna aplikacja nawozu na górną i dolną stronę blaszki liścia. Silniejszy wpływ nawożenia na wszystkie badane parametry obserwowano dla większego natężenia światła.

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