ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 9(3) 2010, 183-190*

# THE EFFECT OF NITROGEN FERTILIZATION ON YIELDING AND BIOLOGICAL VALUE OF SPINACH GROWN FOR AUTUMN HARVEST

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**Abstract.** The nutritional value of some vegetables may greatly vary depending on the cultivation method and kind of fertilization. In 2007–2009 there was conducted a field experiment to evaluate the effects of nitrogen fertilizers (ammonium nitrate, calcium nitrate, ammonium sulphate, urea, Entec-26) and their doses (80, 160 kgN·ha$^{-1}$) on yielding and biological quality of two spinach cultivars (Spokane $F_1$, Rembrandt $F_1$) grown for autumn harvest. The use of Entec-26 fertilizer assured the highest mean marketable yield of spinach (12.25 t·ha$^{-1}$). Comparable results were observed for ammonium sulphate and ammonium nitrate while lower yield was obtained in treatment supplied with calcium nitrate. The highest accumulation of nitrates and vitamin C was associated with the application of calcium nitrate and the lowest one with ammonium sulphate and Entec-26. An increase of the dose of fertilizer from 80 to 160 kg N·ha$^{-1}$ did not significantly affect yielding of spinach and its nutritional value expressed by the content of dry matter, vitamin C and nitrates. Among the two cultivars, Rembrandt $F_1$ produced higher yield of leaves and showed some tendency to a higher accumulation of nitrates, whereas lower content of dry matter and vitamin C. The levels of P, K, Mg and Ca were not dependent on the cultivar as well as kind and dose of nitrogen fertilizer.

**Key words**: cultivars, nutrition, marketable yield, dry matter, nitrates, vitamin C

## INTRODUCTION

Spinach belongs to the group of vegetables characterized by short vegetation period and relatively high growth dynamics [Wojciechowska 2005]. Intensive production associated with application of high doses of fertilizers including in particular nitrogen fertilization contributes to accumulation and in consequence high nitrate content in spinach leaves [Markovic et al. 1988, Pavlovic et al. 1998, Boteva 2009]. Such accumulation can also be affected by microelements, especially molybdenum, which deficits were reported to increase nitrate content [Michalik and Szwonek 1994]. In addition, nitrate

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content highly depends on genetic predispositions of particular species and cultivar, but also on a number of environmental conditions such as the type of soil, its pH or moisture as well as light conditions [Maynard and Barker 1979, Araki et al. 1999, Rożek 2000]. Term of harvest and also the method and storage period may cause the changes of nitrates, nitrites, carotene and vitamin C level in leaves [Bergquist et al. 2005, Wojciechowska 2005]. Excessive accumulation of nitrates in leaves affects the quality of the product. Furthermore, nitrite generated from nitrates in a process of reduction can be at the origin of intoxication or contribute to vitamin A and B deficits [Smoczyński and Skibniewski 1996]. In order to prevent such problems, it is important to select appropriate cultivar of spinach and use a proper types and doses of nitrogen fertilizers [Qingyan et al. 1995, Pavlovic et al. 1998]. Particularly well suited fertilizers are those with slow released nutrients or containing nitrification inhibitors [Maynard and Barker 1979, Rahn 2000, Hähndel and Zerulla 2001]. One of such fertilizers is Entec-26 containing 26% N (7.5% in nitrate form and 18.5% as ammonium salt) and 0.8% of nitrification inhibitor, which stabilizes ammonium form of nitrogen during the period of 4–10 weeks and reduces its loss through leaching. In consequence Entec-26 leads to lower accumulation of nitrates in vegetables.

The main goal of the present study was the evaluation of the nitrogen fertilization effects on the yield and biological quality of spinach grown for autumn harvest.

## MATERIAL AND METHODS

The field experiments were carried out in 2006–2008 in the Experimental Station of the Horticulture Department of the University of Environmental and Life Sciences in Wroclaw. There were studied the effects of two cultivars of spinach including Spokane $F_1$ and Rembrandt $F_1$, five N fertilization sources including ammonium nitrate 34% N, calcium nitrate 15% N, ammonium sulphate 21% N, urea 46% N and Entec – 26% N each applied at two doses (80 and 160 kg N·ha$^{-1}$). The experiment was established according to random blocks method with four replications and plot area 3.6 m$^2$ (1.2×3.0 m). The phosphorus and potassium fertilization in the form of superphosphate and potassium chloride were carried out according to the results of the chemical analysis of the soil in order to obtained final concentration of 60 mg P i 175 mg K in 1 dm$^3$ of the soil. The pH of the soil was 6.7.

The spinach seeds treated with Zaprawa nasienna T (5 g·kg$^{-1}$ seeds) and were sown on the 16$^{th}$, 21$^{st}$ and 20$^{th}$ of August in consecutive years, respectively. The inter-row distance was 20cm and the depth of seeding 3 cm. The seeding was carried out at the rate of 20 kg·ha$^{-1}$.

The harvest of the spinach was conducted on the 16$^{th}$, 27$^{th}$ and 30$^{th}$ of October, respectively. During the harvest 20 plants from each treatment were collected for chemical analysis. Dry matter content was evaluated after drying at 105°C to constant weight, vitamin C by Tillmans' method, phosphorus and magnesium by spectrophotometric method, potassium and calcium using flame photometric method and nitrate nitrogen by potentiometric method.

The results were analyzed using Tuckey's test with confidence coefficient of $\alpha = 0.05$.

## RESULTS AND DISCUSSION

The effects of the nitrogen fertilization on yielding shown in table 1 indicate that the use of Entec-26 produced the highest yield of spinach (12.25 t·ha$^{-1}$). Similar results were obtained using ammonium sulphate and ammonium nitrate, whereas significantly lower crop yield was obtained using calcium nitrate. An increase of the dose from 80 to 160 kg N·ha$^{-1}$ did not significantly affect spinach yielding. Such data are in contrast with those reported by Markovic et al. [1988], Elia et al. [1998], Pavlovic et al. [1998] and Gülser [2005] who observed an increase of the yield in conditions of higher nitrogen doses. Considering the spinach cultivars, significantly highest yielding was found for Rembrandt $F_1$(12.87 t·ha$^{-1}$), which provided the highest crop when combined with ammonium nitrate fertilizer. Similar results were obtained with the use of ammonium sulphate and Entec-26, whereas calcium nitrate was less effective. The best yielding in the entire experiment was found for Rembrandt $F_1$ fertilized with Entec-26 at the dose of 80 kg N·ha$^{-1}$ (14.31 t·ha$^{-1}$). Significantly lowest yielding was observed for Spokane $F_1$ fertilized with calcium nitrate at the dose of 160 kg N·ha$^{-1}$.

Table 1. The effect of nitrogen fertilization on yielding of spinach grown for autumn harvest (mean for 2007–2009)

Tabela 1. Wpływ nawożenia azotem na plon handlowy szpinaku w uprawie na zbiór jesienny (średnio z lat 2007–2009)

| Kind of fertilizer<br>Rodzaj nawozu | Dose of nitrogen<br>Dawka azotu<br>kg·ha$^{-1}$ | Marketable yield – Plon handlowy, t·ha$^{-1}$ | | |
|---|---|---|---|---|
| | | cultivar – odmiana | | mean<br>średnio |
| | | Spokane $F_1$ | Rembrandt $F_1$ | |
| Ammonium nitrate<br>Saletra amonowa | 80 | 10.38 | 13.22 | 11.80 |
| | 160 | 9.77 | 13.58 | 11.67 |
| | $\bar{x}$ | 10.08 | 13.40 | 11.74 |
| Calcium nitrate<br>Saletra wapniowa | 80 | 11.53 | 12.78 | 12.15 |
| | 160 | 9.39 | 10.71 | 10.05 |
| | $\bar{x}$ | 10.46 | 11.74 | 11.10 |
| Ammonium sulphate<br>Siarczan amonu | 80 | 10.65 | 12.67 | 11.66 |
| | 160 | 11.24 | 14.11 | 12.67 |
| | $\bar{x}$ | 10.94 | 13.39 | 12.17 |
| Entec-26 | 80 | 10.63 | 14.31 | 12.47 |
| | 160 | 12.47 | 11.62 | 12.04 |
| | $\bar{x}$ | 11.55 | 12.96 | 12.25 |
| Mean for N dose<br>Średnio dla dawki N | 80 | 10.80 | 13.24 | 12.02 |
| | 160 | 10.71 | 12.50 | 11.61 |
| Mean – Średnio | | 10.76 | 12.87 | 11.81 |

$LSD_{0.05}$, $NIR_{0,05}$:

| | | |
|---|---|---|
| cultivar – odmiana (A) | | 0.92 |
| kind of fertilizer – rodzaj nawozu (B) | | 1.08 |
| dose of nitrogen – dawka azotu (C) | | n.s. – n.i. |
| interaction – interakcja | (A×B) | 1.53 |
| | (A×C) | 1.16 |
| | (B×C) | 1.64 |
| | (A×B×C) | 2.32 |

The dry matter content was not significantly different in the leaves of the two spinach cultivars and ranged between 13.57% for Spokane $F_1$ and 14.47% for Rembrandt $F_1$ (tab. 2). There was not found any significant effect of the kind of fertilizer and the dose of nitrogen on the dry matter content in the leaves. Considering simultaneously all tested treatments the spinach, the use of 80 kg N·ha$^{-1}$ of ammonium nitrate in Spokane $F_1$ cv. provided the highest level of dry matter (16.30%).

Table 2. The effect of nitrogen fertilization on dry matter content of spinach grown for autumn harvest (mean for 2007–2009)

Tabela 2. Wpływ nawożenia azotem na zawartość suchej masy w szpinaku uprawianym na zbiór jesienny (średnio z lat 2007–2009)

| Kind of fertilizer<br>Rodzaj nawozu | Dose of nitrogen<br>Dawka azotu<br>kg·ha$^{-1}$ | Dry matter – Sucha masa, % | | |
|---|---|---|---|---|
| | | cultivar – odmiana | | mean<br>średnio |
| | | Spokane $F_1$ | Rembrandt $F_1$ | |
| Ammonium nitrate<br>Saletra amonowa | 80 | 16.30 | 12.80 | 14.55 |
| | 160 | 14.13 | 13.57 | 13.85 |
| | $\bar{x}$ | 15.22 | 13.18 | 14.20 |
| Calcium nitrate<br>Saletra wapniowa | 80 | 13.87 | 14.20 | 14.03 |
| | 160 | 13.87 | 13.77 | 13.82 |
| | $\bar{x}$ | 13.87 | 13.98 | 13.93 |
| Ammonium sulphate<br>Siarczan amonu | 80 | 14.77 | 13.77 | 14.27 |
| | 160 | 14.23 | 13.03 | 13.63 |
| | $\bar{x}$ | 14.50 | 13.40 | 13.95 |
| Entec-26 | 80 | 14.37 | 13.57 | 13.97 |
| | 160 | 14.20 | 13.87 | 14.03 |
| | $\bar{x}$ | 14.28 | 13.72 | 14.00 |
| Mean for N dose<br>Średnio dla dawki N | 80 | 14.83 | 13.58 | 14.20 |
| | 160 | 14.11 | 13.56 | 13.83 |
| Mean – Średnio | | 14.47 | 13.57 | 14.02 |

$LSD_{0.05}$, $NIR_{0,05}$:

| | | |
|---|---|---|
| cultivar – odmiana (A) | | n.s. – n.i. |
| kind of fertilizer – rodzaj nawozu (B) | | n.s. – n.i. |
| dose of nitrogen – dawka azotu (C) | | n.s. – n.i. |
| interaction – interakcja | (A×B) | 1.44 |
| | (A×C) | 0.94 |
| | (B×C) | n.s. – n.i. |
| | (A×B×C) | 1.88 |

Chemical analysis revealed that, although not statistically significant, Rembrandt $F_1$ showed a tendency to accumulate more nitrates than Spokane $F_1$ (tab. 3). Considering the kind of applied fertilizer, plants fertilized with calcium nitrate had the highest content of nitrates (716 mg $NO_3$·kg$^{-1}$ f.w.). The least nitrates accumulated plants fertilized with ammonium sulpahte (488 mg $NO_3$·kg$^{-1}$ f.w.). Similar tendency was observed by Elia et al. [1998].

An increase of the dose of nitrogen from 80 to 160 kg N·ha$^{-1}$, showed a tendency to increase the accumulation of nitrates. Although such tendency was not statistically significant, such relationship was previously observed by Elia et al. [1998], Markovic et al. [1988], Pavlovic et al. [1998], Gülser [2005] and Boteva [2009]. These reports indicated

Table 3. The effect of nitrogen fertilization on nitrates content of spinach grown for autumn harvest (mean for 2007–2009)

Tabela 3. Wpływ nawożenia azotem na zawartość azotanów w szpinaku uprawianym na zbiór jesienny (średnio z lat 2007–2009)

| Kind of fertilizer<br>Rodzaj nawozu | Dose of nitrogen<br>Dawka azotu<br>kg·ha$^{-1}$ | Nitrates (mg $NO_3$·kg$^{-1}$ f. w.)<br>Azotany (mg $NO_3$·kg$^{-1}$ św. m.) | | |
|---|---|---|---|---|
| | | cultivar – odmiana | | mean<br>średnio |
| | | Spokane $F_1$ | Rembrandt $F_1$ | |
| Ammonium nitrate<br>Saletra amonowa | 80 | 554 | 556 | 555 |
| | 160 | 608 | 801 | 705 |
| | $\bar{x}$ | 581 | 679 | 630 |
| Calcium nitrate<br>Saletra wapniowa | 80 | 538 | 654 | 596 |
| | 160 | 913 | 760 | 836 |
| | $\bar{x}$ | 726 | 706 | 716 |
| Ammonium sulphate<br>Siarczan amonu | 80 | 493 | 662 | 578 |
| | 160 | 415 | 382 | 398 |
| | $\bar{x}$ | 454 | 522 | 488 |
| Entec-26 | 80 | 417 | 599 | 508 |
| | 160 | 587 | 546 | 566 |
| | $\bar{x}$ | 502 | 572 | 537 |
| Mean for N dose<br>Średnio dla dawki N | 80 | 501 | 618 | 559 |
| | 160 | 631 | 622 | 626 |
| Mean – Średnio | | 566 | 620 | 593 |

$LSD_{0.05}$, $NIR_{0,05}$:

| | | |
|---|---|---|
| cultivar – odmiana (A) | | n.s. – n.i. |
| kind of fertilizer – rodzaj nawozu (B) | | 133 |
| dose of nitrogen – dawka azotu (C) | | n.s. – n.i. |
| interaction – interakcja | (A×B) | 188 |
| | (A×C) | n.s. – n.i. |
| | (B×C) | 223 |
| | (A×B×C) | 316 |

also that best yielding cultivars were characterized by the highest tendency to accumulate nitrates. Significant effects of the dose and kind of nitrogen fertilizer on nitrates accumulation was indicated by Rożek [2000] and Wojciechowska [2005]. Considering the effect of fertilizer depending on the spinach cultivar, the highest levels of nitrates contained by Spokane $F_1$ fertilized with calcium nitrate (726 mg $NO_3$·kg$^{-1}$ f.w.), whereas the lowest levels were observed in the same cultivar but fertilized with ammonium sulphate. Non-significantly more nitrates were found in leaves from plots fertilized with Entec-26. The favourable effects this fertilizer on decrease of nitrates content was also observed in leeks, lamb's lettuce, spinach, carrot and lettuce cultivation [Hähndel and Zerulla 2001]. Szura et al. [2008] did not present any effect of ENTEC 26 fertilization on the content of nitrates in red beet leaves and roots, however non-significantly lower content of nitrates were found in plants fertilized with ENTEC 26. Generally, the highest levels of nitrates were determined in Spokane $F_1$ after calcium nitrate application at the dose of 160 kg N·ha$^{-1}$ (913 mg $NO_3$·kg$^{-1}$ f.w.), whereas the lowest one in Rembrandt $F_1$ fertilized with ammonium sulphate at the dose of 160 kg N·ha$^{-1}$. All these amounts remained below the maximum limit of nitrates (2500 mg $NO_3$·kg$^{-1}$) established by the Ministry of Health [Rozporządzenie Komisji WE nr 1882/2005 z dn. 08.11.2005].

Table 4. The effect of nitrogen fertilization on vitamin C content of spinach grown for autumn harvest (mean for 2007–2009)

Tabela 4. Wpływ nawożenia azotem na zawartość witaminy C w szpinaku uprawianym na zbiór jesienny (średnio z lat 2007–2009)

| Kind of fertilizer<br>Rodzaj nawozu | Dose of nitrogen<br>Dawka azotu<br>kg·ha$^{-1}$ | Vitamin C (mg·100 g$^{-1}$ f. w.)<br>Witamina C (mg·100 g$^{-1}$św. m.) | | |
|---|---|---|---|---|
| | | cultivar – odmiana | | mean<br>średnio |
| | | Spokane $F_1$ | Rembrandt $F_1$ | |
| Ammonium nitrate<br>Saletra amonowa | 80 | 92.87 | 85.74 | 89.30 |
| | 160 | 117.65 | 93.03 | 105.34 |
| | $\bar{x}$ | 105.26 | 89.39 | 97.32 |
| Calcium nitrate<br>Saletra wapniowa | 80 | 109.70 | 99.40 | 104.55 |
| | 160 | 124.53 | 123.13 | 123.83 |
| | $\bar{x}$ | 117.12 | 111.27 | 114.19 |
| Ammonium sulphate<br>Siarczan amonu | 80 | 100.47 | 105.43 | 102.95 |
| | 160 | 103.80 | 89.68 | 96.74 |
| | $\bar{x}$ | 102.13 | 97.56 | 99.85 |
| Entec-26 | 80 | 67.53 | 86.87 | 77.20 |
| | 160 | 103.40 | 99.93 | 101.67 |
| | $\bar{x}$ | 85.47 | 93.40 | 89.43 |
| Mean for N dose<br>Średnio dla dawki N | 80 | 92.64 | 94.36 | 93.50 |
| | 160 | 112.35 | 101.45 | 106.90 |
| Mean – Średnio | | 102.49 | 97.90 | 100.20 |

$LSD_{0.05}$, $NIR_{0,05}$:

| | | |
|---|---|---|
| cultivar – odmiana (A) | | n.s. – n.i. |
| kind of fertilizer – rodzaj nawozu (B) | | 18.76 |
| dose of nitrogen – dawka azotu (C) | | n.s. – n.i. |
| interaction – interakcja | (A×B) | 26.53 |
| | (A×C) | n.s. – n.i. |
| | (B×C) | 27.95 |
| | (A×B×C) | 39.53 |

The content of the vitamin C in spinach leaves is shown in table 4. Although there was no statistical difference, Spokane $F_1$ showed a tendency for accumulation more vitamin C than Rembrandt $F_1$. Much lower content of vitamin C in spinach (above 40%) obtained in their research Qingyan et al [1995]. Among the fertilizers investigated in this experiments, the use of calcium nitrate was associated with the highest content of vitamin C (114.19 mg·100 g$^{-1}$ f.w.), whereas the lowest levels of this compound was found in plants fertilized with Entec-26. Increasing nitrogen doses did not significantly affect these amount and the mean vitamin C content in the study was 100.2 mg·100 g$^{-1}$ of fresh matter. The analysis of the effects of fertilizer as a function of cultivar showed that the highest vitamin C content was present in Spokane $F_1$ fertilized with calcium nitrate, whereas the lowest one was observed in the same cultivar after using Entec-26. Generally, Spokane $F_1$ fertilized with calcium nitrate at the dose of 160 kg N·ha$^{-1}$ showed highest vitamin C accumulation (124.53 mg·100 g$^{-1}$ f.w.) in opposition to the use of Entec-26 at the dose of 80 kg N·ha$^{-1}$, which in the same cultivar was associated with the lowest accumulation of vitamin C.

The content of minerals in the leaves of spinach attained on average 0.42% P, 7.42% K, 0.23% Ca, 0.48% Mg. These amounts were not significantly affected by cultivar or the kind of fertilizer.

## CONCLUSIONS

1. The spinach cultivar Rembrandt $F_1$, grown for the autumn harvest was characterized by higher marketable yield than Spokane $F_1$. The type of cultivar did not significantly affect the content of dry matter, nitrates or vitamin C.

2. Among the tested N sources the highest yielding were obtained by using Entec-26. Similar results were obtained in treatment with ammonium sulphate and ammonium nitrate, whereas the least effective appeared to be calcium nitrate, which also provided to the highest amount of nitrates and vitamin C in spinach leaves.

3. Yielding as well as the content of dry matter, nitrates and vitamin C, were not affected by increasing nitrogen doses from 80 to 160 kg $N \cdot ha^{-1}$. However, there was observed some tendency for increment of yield and dry matter content, while the decrease of nitrates and vitamin C in case of heavy nitrogen application.

4. Rembrandt $F_1$ cv. fertilized with Entec-26 at the dose of 80 kg $N \cdot ha^{-1}$ provided the highest marketable yield, whereas the highest nitrate and vitamin C content was found in Spokane $F_1$ fertilized with calcium nitrate at 160 kg $N \cdot ha^{-1}$.

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## WPŁYW NAWOŻENIA AZOTEM NA PLONOWANIE I WARTOŚĆ BIOLOGICZNĄ SZPINAKU W UPRAWIE NA ZBIÓR JESIENNY

**Streszczenie.** Wartość odżywcza niektórych warzyw może w znacznym stopniu różnić się w zależności od metody uprawy i rodzaju nawożenia. W latach 2007–2009 przeprowadzono doświadczenie dotyczące oceny wpływu rodzaju nawozu azotowego (saletra amonowa, saletra wapniowa, siarczan amonu, mocznik, Entec-26) i stosowanej dawki azotu (80, 160 kg $N \cdot ha^{-1}$) na plonowanie i wartość biologiczną dwóch odmian szpinaku (Spokane $F_1$, Rembrandt $F_1$) uprawianego na zbiór jesienny. Użycie nawozu Entec-26 zapewniło przeciętnie najwyższy plon handlowy szpinaku (12,25 $t \cdot ha^{-1}$). Na tym samym poziomie plonowały rośliny nawożone siarczanem amonu i saletrą amonową, podczas gdy najmniejszy plon stwierdzono po zastosowaniu saletry wapniowej. Najmniej azotanów i witaminy C gromadziły rośliny nawożone siarczanem amonu i nawozem Entec-26. Wzrost dawki azotu z 80 do 160 kg $N \cdot ha^{-1}$ nie miał istotnego wpływu na wysokość plonu szpinaku oraz zawartości suchej masy, witaminy C i azotanów. Spośród badanych odmian wyższym plonem i większą tendencją do gromadzenia azotanów oraz mniejszą zawartością suchej masy i witaminy C odznaczała się odmiana Rembrandt $F_1$. Poziom P, K, Mg i Ca nie był w istotny sposób zależny od odmiany, rodzaju oraz dawki azotu.

**Słowa kluczowe**: odmiany, nawożenie, plon handlowy, sucha masa, azotany, witamina C

Accepted for print – Zaakceptowano do druku: 23.06.2010