# IMPACT OF INCREASING NITROGEN RATES ON THE COURSE OF THE NITROGEN CRITICAL CONCENTRATION CURVE DURING THE VEGETATIVE GROWTH OF WINTER WHEAT

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#### Abstract

Nitrogen is the dominant factor affecting the rate of wheat growth and yielding. Water supply during the critical stages of yield component formation is a factor limiting nitrogen use efficiency. A study dealing with this problem has been conducted, based on a long-term field experiment with four N levels (0, 60, 120, 180 kg N ha<sup>-1</sup>) against the background of four systems of potassium management, including: medium and high K soil fertility levels without and with K fertilizer (MK-, MK+, HK-, HK+). The objective of the study was to evaluate the wheat nitrogen nutritional status during the vegetative period of wheat growth according to nitrogen concentrations in leaves and stems. The research was run in 2003, with severe deficit of water, and in 2004, under semi-dry water conditions. Grain yield of wheat responded to both experimental factors only in 2004. Nitrogen concentration in plant parts was dependent only on N doses, thus underlying good adaptation of wheat to the seasonal course of water supply. Nitrogen concentration in leaves followed the quadratic regression model. This type of response indicates an N saturation status, i.e. a non-limiting effect of this factor on plant growth. The pattern of N concentration in stems was in accordance with the linear regression model. This type of response indicates the N-limited growth due to an insufficient supply of nitrogen. The Critical Nitrogen Concentration (CNC) pattern showed significant adaptability of wheat to N fertilizer levels. The Nitrogen Nutrition Index, calculated from the CNC, can serve as an indicator of N dilution during the vegetative period of wheat growth.

Keywords: nitrogen concentration, plant organs, vegetation, dilution effect, wheat.

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#### WPŁYW WZRASTAJĄCYCH DAWEK AZOTU NA PRZEBIEG KRZYWEJ KRYTYCZNEJ KONCENTRACJI AZOTU W PSZENICY OZIMEJ W OKRESIE WZROSTU WEGETATYWNEGO

#### Abstrakt

Azot jest czynnikiem dominującym, kształtującym szybkość wzrostu pszenicy i plonowanie. Zaopatrzenie rośliny w wodę w krytycznych fazach formowania elementów struktury plonu jest głównym czynnikiem ograniczającym efektywność azotu. Problem ten badano w ramach wieloletniego doświadczenia statycznego, stosując 4 poziomy nawożenia azotem (0, 60, 120, 180 kg N ha<sup>-1</sup>) na tle 4 systemów gospodarki potasem, w tym: o średnim i wysokim poziomie zasobności w przyswajalny azot oraz bez aplikacji potasu lub z bieżąca jego aplikacją (MK-, MK+, HK-, HK+). Celem badań była ocena stanu odżywienia pszenicy azotem w okresie wzrostu wegetatywnego. Badania prowadzono w 2003 r. o dużych niedoborach wody i 2004 r. - z niedoborami okresowymi. Plony pszenicy ozimej wykazały reakcję na oba czynniki doświadczalne tylko w 2004 roku. Koncentracja azotu w częściach rośliny zależała tylko od dawek azotu, co świadczy o dużej zdolności adaptacyjnej pszenicy do reżimu pogodowego w okresie wegetacji. Koncentracja azotu w liściach przebiegała zgodnie z kwadratowym modelem regresji. Taki typ reakcji na wzrastające dawki azotu wskazuje na stan wysycenia rośliny-organu azotem, co oznacza brak ograniczającego działania tego czynnika wzrostu. Natomiast model koncentracji azotu w źdźbłach był zgodny z modelem regresji liniowej. Ten model reakcji rośliny-organu wskazuje na ograniczenie wzrostu, wywołane niedostatecznym zaopatrzeniem w azot. Krzywa krytycznej koncentracji azotu (KKKN) wykazała bardzo dużą plastyczność reakcji pszenicy na dawki nawozowe azotu. Indeks odżywienia azotem (ION), obliczony na podstawie KKKN, może być zastosowany jako wskaźnik rozcieńczenia azotu w okresie wzrostu wegetatywnego pszenicy.

Słowa kluczowe: zawartość azotu, organy rośliny, wegetacja, efekt rozcieńczenia, pszenica.

# INTRODUCTION

Nitrogen supply to growing plants, due to its strong impact on dry matter partitioning among plant organs, is considered as the main growth factor (RUBIO et al., 2003). Nitrogen uptake rate depends on both its concentration in the soil solution, soil water content, and plant uptake potential as related to its nutritional status (FORDE, LORENZO 2001). The key challenge for agronomists is to reach during the crucial stages of yield component's formation the required range of nitrogen concentration. Nitrogen concentration in a plant during its life-cycle is not constant, changing in accordance to stage of growth, showing a specific dilution pattern for cereals (FABER 2004, BARTCZAK 2008), maize (GRZEBISZ et al., 2008). The principal reason for this phenomenon, is dry matter redistribution between metabolic, photosynthetic active parts, and structural -photosynthetic passive parts. The main reason for N dilution is a much faster growth rate of constitutional versus leaves during the vegetative period of the life-cycle. The concept of Critical Nitrogen Concentration (CNC) assumes that maximum biomass production in a defined environment requires an adequate N concentration in plant tissues (LEMAIRE et al. 2008). The general relationship between both components is described using the power function:

 $N_a = a D M^{-b}$ ,

where:

 $N_c$  – critical nitrogen concentration (% DM), DM – yield of dry matter (t ha<sup>-1</sup>), a, b – coefficients.

A specific role in each crop response to water and nitrogen stresses is attributed to potassium. Under ample-water condition, the mass-flow of nitrate-nitrogen towards the roots is the main route of plant requirement's covering. The hormone signaling cascade has been recently considered as the key factor changing the route of assimilates partitioning among plant organs in response to decline nitrogen-nitrate concentration in the soil solution on one side and the systemic response of aboveground parts on the other (DE-BAEKE, ABOUDRARE 2004, GONZALEZ-DUGO et al. 2010). Potassium is involved in plant response to water stress. This nutrient undergoes a constant circulation among plant parts, being responsible for new tissue's growth, including roots. Therefore, plants well supplied with potassium are capable to develop fresh roots, in turn exploring soil patches rich in water and nitrogen. Consequently, it is assumed that plant crops well-supplied with potassium are able to overcome to some extend a water stress due to increased uptake of soil nitrogen (GRZEBISZ et al. 2013).

The main objective of the study was to validate the concept of critical nitrogen concentration on the background of progressive nitrogen fertilizer rates and four systems of potassium fertilization in winter wheat during its vegetative growth.

### MATERIALS AND METHODS

Study on winter wheat response to increasing nitrogen rates on the background of four potassium systems were carried out during two consecutive growing seasons 2002/03 and 2003/04 at Brody (Experimental Station of the Poznan University of Life Sciences). The long-term experimental trial was established in 1991 on a soil originated from a loamy sand underlined by a light loam soil, classified as Albic Luvisol. Three-factorial, split-block experiment, replicated four times, included following factors:

- 1. Soil potassium fertility level: medium, M and high, H;
- 2. Fresh potassium application: K- (without potassium) and K+ (100 kg K ha<sup>-1</sup>);
- 3. Four rates of nitrogen: 0, 60, 120 and 180 kg N ha<sup>-1</sup>.

The tested systems of potassium fertilization, comprising two first factors are as follows: i) medium without fresh potassium application (acronym MK-), ii) medium with fresh potassium application (MK+), iii) high without fresh potassium application (HK-), high with fresh potassium application (HK+).

Each year maize was a preceding crop for wheat. The size of the main plots was 53.4 m<sup>2</sup> and the individual plot of 13.35 m<sup>2</sup>. The variety *Zyta* was sown in the last decade of September. Phosphorus and potassium were applied prior to sowing in rates adjusted to the soil test values and treatment. Phosphorus was applied in the form of triple super phosphate and potassium as potassium chloride. Nitrogen (ammonium saltpeter) was applied at equal rates of 60 kg N ha<sup>-1</sup> in accordance to the experimental design, i.e., i) before Spring regrowth ii) at the end of tillering, iii) at the stage of the flag leaf appearance. At maturity, crops were harvested from the area of 8.4 m<sup>2</sup> using a plot combine harvester. Total grain yield was adjusted to 14% moisture content.

Plants for assessment of dry mater dynamics were sampled from an area of 1 m<sup>2</sup> in three consecutive stages of wheat growth according to the BBCH scale: 31, 37, and 59. At each sampling date, the harvested plant sample was partitioning, in accordance to stage of development, into sub-samples of leaves, stems, and ears then dried (65°C). Finally, at each stage total and sub-sample dry matter per 1 m<sup>2</sup> was recorded.

The French and Schulz's approach of the water limited yield (WLY), modified by GRZEBISZ et al. (2013) into a graphical form, was applied to discriminate yield fraction dependent on the volume of transpired water, and those induced by potassium fertilizer. The algorithms for the water limited yield (WLY) calculation was as follows:

$$WLY = TE (R - \Sigma E_s) + WR,$$

where:

TE refers to the transpiration efficiency (TE = k/VPD;

 $k \qquad - \ biomass/transformation \ ratio;$ 

VPD – vapor pressure deficit);

- R the sum of rainfall during the growth period;
- $E_s$  the seasonal soil evaporation, equals to 110 mm;
- WR water reserves in the rooted soil volume at the beginning of growth of a particular crop.

Nitrogen concentration in all samples was determined by standard macro-Kjeldahl procedure. In experimental practice the critical nitrogen concentration,  $N_{e}$ , is obtained by plotting data on of empirically determined concentration N (% N in DM) in consecutive stages of plant crop growth *vs.* accumulated shoot biomass DM (t ha<sup>-1</sup>). The existing relationship is classically described by a mononomial function of biomass DM:

$$N_{c} = \alpha DM^{-b},$$

where:

 $N_c$  – critical N concentration (% DM), DM – dry matter yield (t ha<sup>-1</sup>), a and b – coefficients of equation.

The obtained  $N_c$  values are basic set of data for nitrogen nutrition index (NNI) calculation, which presents relative status of plant nitrogen (LEMAIRE, GASTAL 1997):

### NNI = actual N concentration/critical N concentration

The obtained NNIs are expressed as the relative value. Therefore, the NNI index above 1.0 indicates non N-limiting plant crop canopy growth, whereas those below 1.0 reflect N deficiency.

The experimentally obtained data sets were subjected to conventional analysis of variance for the split-plot-plot design. The least significant difference values (LSD at  $P \leq 0, 05$ ) were calculated to establish the significance of mean differences. The simple regression model was applied to determine some relationships between the studied plant characteristics.

## **RESULTS AND DISCUSSION**

### Growing conditions and water productivity

The study aimed at nitrogen impact on the seasonal course of critical nitrogen concentration in wheat plants were conducted in two consecutive seasons. Both years significantly differed in weather during spring vegetation (Table 1). The first, 2003, was dry. The whole sum of precipitation from

Table 1

Months	Sum of precipitation		Long-term	Mean ten	Long-term	
	2003	2004	average	2003	2004	average
January	60.2	73.2	36.0	-1.6	-3.5	-1.9
February	74.0	32.4	29.8	-3.2	2.2	-0.8
March	19.9	20.9	38.0	3.4	5.1	2.7
April	21.1	23.3	38.6	8.2	10.0	7.6
May	20.1	44.3	56.2	16.0	13.6	12.9
June	35.0	58.8	66.5	19.8	16.3	16.2
July	96.7	59.6	78.7	19.6	17.3	17.7

Characteristics of meteorological conditions during spring vegetation of winter wheat\*

\* The Synoptic Station Brody



yield of grain, (t ha<sup>-1</sup>)



the third decade of March to the first decade of July amounted to 116.9 mm. In 2004, total sum of precipitation was slightly higher, amounting to 164.4 mm. The total sum of evapo-transpirated water amounted to 447 and 425 mm, in 2003 and 2004, respectively (calculation based on BROUWER, HEIBLOEM 1986). In spite of relatively small differences in this factor, grain yield response to experimental treatments was year-to-year variable. In 2003, the theoretical yield, defined as Water Limited Yield (WLY) was calculated at the level of 5.258 t ha<sup>-1</sup> and in 2004 at 6.204 t ha<sup>-1</sup>. The real average yield of wheat in 2003 amounted to 3.2 t ha<sup>-1</sup> in the control plot and 4.5 t ha<sup>-1</sup> in the treatment fertilized with 120 kg N ha<sup>-1</sup>. The harvested yield, in spite of response to fertilizer N, was much below the Water Limited Yield (WLY) – Figure 1. The yield gap due insufficient precipitation was not overcome, even creating favorable conditions for nutrients supply. In 2004, maximum yields were about 8 t ha<sup>-1</sup>, above the WLY. The yield gain was due to significant impact of nitrogen and potassium on water productivity. In treatments without applied nitrogen, water productivity, as expected was below the WLY. A slightly higher yield loss was noted for high fertility soil. There has also been noted an inefficient water use in all plots fertilized with 60 kg N ha<sup>-1</sup>. The optimum conditions for water productivity have revealed, irrespectively of K soil fertility level, in treatments with 120 kg N ha<sup>-1</sup> provide a fresh application of fertilizer potassium (K+). These results corroborate the hypothesis formulated by GRZEBISZ et al. (2013) that only a mild-water stress a plant crop response positively to ample supply of potassium (high K soil fertility and/or K fresh application).

#### Dry matter yield of wheat in critical stages vegetative growth

Trends of dry matter yield have been evaluated in three crucial stages of wheat growth, i.e., 31, 37 and 59 in accordance to the BCCH scale. The initial one can be considered as a borderline between the exponential and linear biomass growth pattern (YIN et al. 2003). In general, it can be used to make the first evaluation of cereals nutritional status. The second begins the period of an ear growth, being crucial in the number of ears per unit area and spikelets per plant (ZERCHE, HECHT 1999). The third one, ending the heading phase, finishes the vegetative period. In the conducted study, patterns of leave's growth, as related to their biomass, were governed by two factors, i.e., the course of the weather during each of growth seasons and the rate of applied nitrogen (Table 2). Yield of leaves, irrespectively of the year, as recorded in BBCH 31 and 37, increased in accordance to progressing N rates. At the end of vegetative phase biomass of leaves (YL) followed the quadrate regression functions:

2003: YL2003 =  $-0.0013N^2 + 0.488N + 56.59$  for  $R^2 = 0.94$ ; 2004: YL2004 =  $-0.0048N^2 + 1.453N + 9.96$  for  $R^2 = 0.97$ .

The presented patterns implicitly indicate on nitrogen availability during wheat vegetative growth as the main limiting factor for biomass of

Experimental	Level	2003 (BBCH)			2004 (BBCH)			
factors	of factor	31	37	59	31	37	59	
	М	84.03	80.4	76.55	146.5	152.8	167.2	
Soil fertility level for K**	Н	90.97	118.6	91.97	162.2	165.6	167.6	
	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.	
	K-	94.28	100.6	86.19	173.2	169.3	171.7	
Potassium fertilizing***	K+	80.72	98.38	82.32	135.5	149.0	163.1	
3	LSD 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	0	59.06	67.68	58.56	96.22	93.44	100.6	
	60	115.9	104.0	75.22	212.5	164.8	156.0	
N rates, (kg ha <sup>-1</sup> )	120	-	126.9	102.6	-	219.3	213.4	
(	180	-	-	100.6	-	-	200.0	
	LSD 0.05	17.10	32.59	12.63	23.97	32.33	27.46	

Statistical evaluation of main factors affecting biomass of leaves of winter wheat (g m<sup>-2</sup>)

\* non significant;

\*\* M – medium, H – high soil K fertility level;

\*\*\* K- – potassium control, K+ – 100 kg K ha-1;

leaves. On average, wheat in 2003 produced only 50% of leaves yield as compared to 2004. In accordance to VOILLOT and DEVIENNE-BARRETT (1999) this drastic reduction can be explained by reduction in N supply from soil resources to growing wheat tissues. The authors of the cited paper showed that N remobilization from the  $3^{rd}$  uppermost leaf started at the end of 3-week lasting shortage of N supply. In the presented study the effect of potassium fertilizing systems was non-significant in both years. However, a positive trend of the high potassium fertility on biomass of leaves was observed during the vegetative period in 2003 and at BBCH 31 and 37 in 2004.

An analysis of dry matter yield of stems clearly documents the water stress negative impact on wheat growth during the period of ear growth, i.e., from BBCH 37 to 59. This conclusion is drowned based on biomass yield of stems in 2003 and 2004 at the end of the vegetative period. In 2003, it amounted to 300 g m<sup>-2</sup>, but in 2004, it was three times higher, amounting to 900 g m<sup>-2</sup>. The importance of this particular period for development of yield components is well described in literature. This huge difference implicitly indicates on reduction in the number of tillers in 2003. This conclusion is supported by BAQUE et al. (2006) and GRZEBISZ et. al. (2009) who showed for wheat, that the numbers of tillers are the first component affected by drought stress. Effect of increasing nitrogen rates (N) on the yield of stems (YS) followed the same pattern as found for leaves:

1) 2003:	$YS = -0.006N^2 + 1.67N + 241.8$	for $R^2 = 0.96$ ;
2) 2004:	$YS = -0.019N^2 + 5.61N + 642.5$	for $R^2 = 0.99$ .

As a result of drought in 2003, yield of stems, averaged over years, was lower as compared to 2004, but the relative differences were much smaller. The same pattern of yield of ears (YE) to increasing N rates was found:

2003:	$YE = -0.0023N^2 + 0.815N + 99.0$	for $R^2 = 0.96$ ;
2004:	$YE = -0.0059N^2 + 1.786N + 146.6$	for $R^2 = 0.99$ .

The course of both curves reveals also the fact, that efficiency of applied nitrogen increased both quantitatively and relatively much faster in 2004. Effect of the potassium system was low, revealing as a positive trend in the high potassium fertile soil. In both years, it was slightly stronger in early stages of wheat growth. It can be explained by plant's ability to take potassium from its non-exchangeable resources (GRZEBISZ, OERTLI 1994, KUCHENBUCH et al. 1986).

### Nitrogen concentration in wheat parts

Nitrogen concentration in leaves is frequently used as an indicator of plant nutritional status. The observed differences in its plant characteristic were significant only for nitrogen treatments. It was observed, nitrogen concentration in leaves increased progressively with N rate. This trend was not significant only in 2004 at BBCH 59. Impact of seasons on this wheat attribute was quite specific. Water stressful conditions, as in 2003, significantly affected nitrogen concentration in leaves, which increased from BBCH 31 to BBCH 37, decreasing afterwards. However, in semi-dry 2004, the constant dilution trend was observed in 2004, even accelerating from BBCH 37 to 59. This phenomenon is inversely related to biomass of stems in consecutive stages of wheat growth, i.e., yields of structural parts of the wheat plant (Table 3). This morphometric process can be explained by two facts. The growing stem, including the ear is a big physiological sink for nitrogen. Its supply during the period of the ear growth is decisive both for assimilates production by leaves and the number of spikelets (GRZEBISZ 2013, SHEARMAN et al. 2005, ZERCHE, HECHT 1999).

Nitrogen concentration in stems, averaged over all other treatments, was usually higher in the dry 2003, compared to the semi-dry 2004 (Table 4). It showed a declining trend during vegetative growth. In addition, its rate was much slower in the dry 2003 than in normal 2004. Nitrogen concentration in stems (SN) responded to increase N rates (N) following the linear regression model:

2003: SN = 0.025N + 7.65 for  $R^2 = 0.96$ 2004: SN = 0.0127N + 7.21 for  $R^2 = 0.99$ .

The rate of N concentration in stems in response to increasing N rates was twice as high in the dry 2003 compared to 2004. This attribute of developed equations clearly explains the higher yield of stems in 2004.

Nitrogen concentration of N in developed ears (NE), evaluated just before flowering, was almost the same in both years. However, it response to increasing nitrogen rates (N), was described by distinct regression models:

Table 3

Experimental factors		2003 (BBCH)			2004 (BBCH)		
	Level of factor	ste	ems	ears	ste	ms	ears
1400015		37	59	CH)     20       ears     ster       59     37       139.1     281.9       146.7     323.2       n.s.     28.3       143.9     305.7       141.9     299.4       n.s.     n.s.       101.5     173.8       131.9     344.3       167.6     -       18.63     75.68	59	59	
	М	199.1	300.0	139.1	281.9	904.4	232,1
Soil fertility	Н	242.2	339.3	146.7	323.2	909.4	232.7
	LSD 0.05	n.s.*	n.s.	n.s.	28.3	n.s.	n.s.
	K-	222.2	327.7	143.9	305.7	921.1	227.1
Potassium fertilizing***	K+	219.1	311.6	141.9	299.4	892.7	237.7
loi unizing	LSD 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	0	151.8	246.0	101.5	173.8	634.6	148.7
	60	239.2	308.2	131.9	344.3	934.2	225.9
N rates, (kg ha <sup>-1</sup> )	120	270.9	371.8	170.6	389.4	1017.1	281.7
(	180	-	352.6	167.6	-	1041.6	273.3
	LSD 0.05	39.36	39.99	18.63	75.68	170.7	46.97

Statistical evaluation of main factors affecting biomass of stems and ears of winter wheat (g m<sup>2</sup>)

\* non significant,

\*\* M – medium, H – high soil K fertility level, \*\*\* K- – potassium control, K+ – 100 kg K ha<sup>-1</sup>;

Table 4

in stems and ears of whiter wheat (g kg Din)							
Experimental		2003 (BBCH)			2004 (BBCH)		
	Level of factor	ste	ms	ears	stems		ears
		37	59	59	37	59	59
	М	13.08	10.03	18.58	13.41	8.466	18.37
Soil fertility level for K**	Н	12.86	9.757	17.61	14.00	8.214	17.89
level lot IX	LSD 0.05	n.s.	n.s.	0.832	n.s.	n.s.	n.s.
	K-	12.97	9.636	17.75	13.79	8.252	17.66
Potassium fertilizing***	K+	12.97	10.15	18.44	13.63	8.428	18.61
forthing	LSD 0.05	n.s.	n.s.	n.s*	n.s.	n.s.	n.s.
	0	11.11	7.882	16.59	12.12	7.187	15.56
	60	12.29	8.571	17.57	12.88	7.856	17.86
N rates, (kg N ha <sup>-1</sup> )	120	15.51	10.98	18.64	16.12	8.949	19.69
	180	-	12.14	19.58	-	9.368	19.42
	LSD 0.05	1.327	0.593	1.198	1.476	1.257	1.653

### Statistical evaluation of main factors affecting nitrogen concentration in stems and ears of winter wheat $(g \ kg^1 DM)$

\* non significant,
\*\* M – medium, H – high soil K fertility level,
\*\*\* K- – potassium control, K+ – 100 kg K ha<sup>-1</sup>;

2003:	NE = 0.0167N + 16.6	for $R^2 = 1$ ;
2004:	$NE = 0.0002N^2 + 0.056N + 15.43$	for $R^2 = 0.99$ .

The first linear, implicitly indicates on nitrogen supply as the factor limiting nitrogen concentration in fully developed ears. In 2004, it revealed, as "the N saturation model," which underlined an ample supply of nitrogen to growing ears.

#### Critical nitrogen concentration course

The physiological term, the critical nitrogen concentration  $(N_c)$ , defines the minimum concentration of N in the plant during its vegetative growth as a prerequisite of for the highest biomass. The course of  $N_c$  during the growing season is termed as the Critical Nitrogen Dilution Course (CNDC). It is developed by plotting pairs, comprising concentration of  $N_c$  (% DM) and respective biomass of aboveground plant parts (DM, t ha<sup>-1</sup>). As presented in Tables 2-6 nitrogen fertilizer rate was the only factor significantly affected both plant biomass, and N concentration in wheat parts. Therefore, the CNDCs were calculated for each N treatment, based on all data for the responsive plot.

The N<sub>c</sub> course during the vegetative period of wheat growth is well described by a power function. The key differences, resulting from the impact of fertilizer nitrogen rate, are attributed to both constants, i.e., "a" and "b". The first one informs about initial N concentration in wheat tissue, and the second one describes steepness of a curve over the course of vegetation, in Table 5

Experimental factors Soil fertility level for K** Potassium fertilizing***	Level	2003 (BBCH)			2004 (BBCH)			
	of factor	31	37	59	31	37	59	
	М	27.70	29.67	25.89	22.26	21.39	12.04	
Soil fertility level for K**	Н	29.52	30.96	25.54	23.38	22.70	11.69	
	LSD 0.05	1.527	n.s.	n.s.	n.s.	n.s.	n.s.	
	K-	28.86	30.01	24.85	22.74	21.55	12.01	
Potassium fertilizing***	K+	28.46	30.62	26.58	22.90	22.54	11.72	
101 United by	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.	
	0	25.64	26.59	20.65	19.55	16.76	10.75	
	60	31.58	29.13	22.71	26.08	20.54	11.79	
N rates,	120	-	35.23	28.44	-	28.83	12.82	
	180	-	-	31.05	-	-	12.10	
	LSD 0.05	2.005	2.088	1.864	2.830	2.370	n.s.	

 $\begin{array}{l} Statistical \mbox{ evaluation of main factors affecting nitrogen concentration} \\ \mbox{ in leaves of winter wheat } (g \ kg^{-1} \ DM) \end{array}$ 

\* non significant,

\*\* M – medium, H – high soil K fertility level,

\*\*\* K- – potassium control, K+ – 100 kg K ha-1;

#### Table 6

Experimental factors		2003			2004		
	Level of factor	N (%DM)	N (% ĎM)	NNI	N (%DM)	N (% D <sup>c</sup> M)	NNI
	М	2.77	3.40	0.84	2.23	2.84	0.79
Soil fertility level for K**	Н	2.95	3.29	0.92	2.34	2.68	0.88
10 / 01 101 11	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.
	K-	2.88	3.26	0.91	2.27	2.84	0.81
Potassium fertilizing***	K+	2.85	3.43	0.85	2.29	2.68	0.86
g	LSD 0.05	n.s.	n.s.	n.s.	n.s.	0.13	n.s.
N rates, (kg ha <sup>-1</sup> )	0 60	$2.56 \\ 3.16$	$3.70 \\ 2.99$	$\begin{array}{c} 0.70\\ 1.06 \end{array}$	$1.96 \\ 2.61$	$\begin{array}{c} 2.48\\ 3.03\end{array}$	$0.79 \\ 0.88$
	LSD 0.05	0.20	0.18	0.07	0.28	0.17	n.s.

Statistical evaluation of main factors affecting nitrogen status in winter wheat at BBCH 31

\* non significant,

\*\* M – medium, H – high soil K fertility level,

\*\*\* K- – potassium control, K+ – 100 kg K ha<sup>-1</sup>;

fact, speed of N dilution. Therefore, in can be concluded that the developed NCDCs reflected fairly well the degree of N supply to wheat plants. Those grown in the N control plot were fully dependent on soil N resources. Consequently, the "a" constant was low, but the  $R^2$  coefficient achieved the highest value, indicating a low N variability, in spite of different growth conditions (Figure 2a). In other three treatments, with increasing N rates, from 60 to 180 kg N ha<sup>-1</sup>, the "a" coefficient was in the narrow range, from 3.04 to 3.12 (Figs. 2b-d). The obtained values of this coefficient are, in fact, low. The main differences between the published CNDC equations refer both to "a" and "b". With respect to the "a" coefficient it ranges from 4.64 (FABER 2004) to 5.07 as presented by (SZCZEPANIAK 2008) up to 5.35 as presented



Fig. 2a. The relationships between dry matter yield and nitrogen concentration - nitrogenous control



Fig. 2b. The relationships between dry matter yield and nitrogen concentration - plot with sub-optimal N rate:  $60 \text{ kg N ha}^{-1}$ 



Fig. 2c. The relationships between dry matter yield and nitrogen concentration - plot with optimal N rate:  $120 \text{ kg N ha}^{-1}$ 



Fig. 2d. The relationships between dry matter yield and nitrogen concentration - plot with supra-optimal N rate:  $180~{\rm kg}~{\rm N}~{\rm ha}^{-1}$ 

by (Justus et al. 1994). The second coefficient "b" varied from -0.36 for the control plot through -0.44 for the 60 kg N ha<sup>-1</sup> treatment to -0.30 for treatments with optimum N productivity. Based on these sets of data it can be concluded that the highest steepness of the NCDC the highest N dilution, indirectly underlying limited N supply. It was the key of attribute of the 60 kg N ha<sup>-1</sup> treatment. For many years, the developed NCDC formula and its coefficients were considered as highly conservative (JUSTUS et al. 1994, LEMAIRE, GASTAL 1997). Eleven years later, LEMAIRE et al. (2008) added to this definition a part "..in a given environment," indicating flexibility of the N<sub>e</sub> to supply of nitrogen. It is worthy to mention that N supply does not depend only on N fertilizer rate. This study fully corroborates this opinion. The obtained "a" value of 3.1, averaged over-all N fertilized treatments, reflects N status of wheat producing yield of grain ranging from 4.0 to 8.0 t ha<sup>-1</sup>, taking into account both dry 2003 and semi-dry 2004 years (Figure 1). In order to explain wheat canopy nutritional status a Nitrogen Nutrition Index (NNI) has been calculated. The NNI value of 1.0 or larger indicates non-limiting, whereas NNI below 1.0 correspond to N deficiency, i.e., N-limiting growth. The evaluation of wheat nutritional status has been conducted for consecutive stages of wheat growth, based on  $N_{c120}$  as the optimal. At the beginning of stem elongation (BBCH 31), the N<sub>c</sub> values were both affected by N rate (N =  $60 \text{ kg N} \text{ ha}^{-1}$ ) and year. In 2003, plants grown on the control plot showed at BBCH 31 a very high value of N concentration of 3.7%, whereas in 2004, it reached only2.5% DM (Table 6). At the same time any impact on  $N_c$  (ca 3% DM) were found in treatments fertilized with 60 kg N ha<sup>-1</sup>. This is the first indicator of N dilution, which was much faster Table 7

Experimental factors		2003			2004		
	Level of factor	N (%DM)	N (% D <sup>c</sup> M)	NNI	N (%DM)	N (% D <sup>c</sup> M)	NNI
	М	1.78	2.30	0.80	1.62	2.02	0.83
Soil fertility	Н	1.87	2.13	0.90	1.70	1.98	0.89
	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.
	K-	1.81	2.22	0.85	1.66	1.98	0.87
Potassium fertilizing***	K+	1.84	2.21	0.85	1.66	2.02	0.85
8	LSD 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	0	1.58	2.45	0.65	1.38	2.31	0.59
N rates,	60	1.73	2.15	0.82	1.54	1.90	0.82
(kg ha-1)	120	2.16	2.04	1.08	2.07	1.79	1.16
	LSD 0.05	0.17	0.10	0.12	0.16	0.15	0.11

Statistical evaluation of main factors af	fecting nitrogen stat	tus in winter wheat at BBCH 37
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\* non significant,

\*\* M - medium, H - high soil K fertility level,

\*\*\* K- – potassium control, K+ – 100 kg K ha<sup>-1</sup>;

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Table 8

Experimental factors	Level of factor	2003			2004		
		%N	NC	NNI	%N	NC	NNI
Soil fertility level for K**	М	1.47	1.89	0.79	1.07	1.42	0.76
	Н	1.43	1.82	0.80	1.04	1.43	0.74
	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.
Potassium fertilizing***	K-	1.41	1.84	0.78	1.04	1.41	0.75
	K+	1.49	1.87	0.81	1.07	1.43	0.76
	LSD 0.05	0.076	n.s.	n.s.	n.s.	n.s.	n.s.
N rates, (kg ha <sup>-1</sup> )	0 60	$1.19 \\ 1.30$	$2.03 \\ 1.88$	$0.59 \\ 0.70$	$0.90 \\ 1.01$	$1.59 \\ 1.42$	$0.60 \\ 0.72$
	120	1.58	1.75	0.91	1.16	1.34	0.86
	180	1.72	1.77	0.98	1.16	1.34	0.87
	LSD 0.05	0.07	0.08	0.06	0.10	0.08	0.09

Statistical evaluation of main factors affecting nitrogen status in winter wheat at BBCH 61

\* non significant,

\*\* M – medium, H – high soil K fertility level,

\*\*\* K- – potassium control, K+ – 100 kg K ha<sup>-1</sup>;

in 2004 than in dry 2003. As a result, the NNI indices for this treatment were close to 1.0 in 2003, whereas below in 2004. In the second evaluated stage of wheat growth, i.e., BBCH 37, values of  $N_c$  were much lower as compared to the preceded stage and responded only to N rate.

Its values, in spite of different course of weather, were almost the same. This result can be explained by stabilization of canopy structure (see Tables 2 and 3). Patterns of  $N_c$  showed an opposite trend to increasing N rates, but the dilution effect was much faster in 2004 than 2003. Quite opposite trend was noted for NNI indices. They were in accordance to N rate, showing, however, a big impact of consecutive N rates. At the end of wheat vegetative growth, i.e., at BBCH 59,  $N_c$  values were slightly lower as compared to the BBCH 37, corroborating the declining trend over the course of vegetation.

The N dilution effect as presented in the previous stage was also pronounced. It was, however, much steeper in 2004 as compared to 2003. The opposite trend was observed for NNI indices, i.e., which increased in accordance to progressing N rates. However, it did not reach the optimum value, even in the plot with 180 kg N ha<sup>-1</sup>.

# CONCLUSIONS

1. Winter wheat response to freshly applied potassium fertilizer can reveal only under conditions of mild-water stress as found in 2004.

2. Nitrogen concentration decline in leaves during the ongoing wheat development is slower, i.e., conservative in dry, compared to semi-dry growth conditions, when undergoes dilution due to higher increase of dry matter yield.

3. Nitrogen concentration in leaves in response to increasing N fertilizer rates follows as a rule the quadrate regression level. In stems dominates the linear model, indicating the N-limited growth conditions.

4. The Critical Nitrogen Concentration pattern in wheat plants shows in response to increasing N rate a significant flexibility during the vegetative period of growth.

5. The Nitrogen Nutrition Index course during wheat growth and development could be used as an indicator of N dilution.

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