

The effect of post-anthesis water supply on grain nitrogen concentration and grain nitrogen yield of winter wheat

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ABSTRACT

The effect of water supply during grain growth on grain nitrogen concentration (GNC) and grain nitrogen yield (GNY) of winter wheat (*Triticum aestivum* L.) was studied in the field experiment on fertile loamy-clay soil in years 2004–2007. The water regime was differentiated using mobile rain shelter (water shortage, treatment S) and drip irrigation (ample water supply, treatment W); rain-fed crop served as the control treatment (R). Wheat was grown without addition of nitrogen and with 200 kg N/ha (N0 and N1, resp.). The effect of water supply on GNC was highly significant ($P < 0.001$) in fertilized wheat and not significant in N0. Drought significantly increased GNC in comparison with irrigated and rain-fed crop in N1. Average grain nitrogen concentrations in respective treatments S, R and W were 1.52, 1.54 and 1.56% in N0 and 2.50, 2.24 and 2.07% in N1. Water availability also significantly affected grain nitrogen yield ($P < 0.01$). The GNY of fertilized wheat under water shortage was significantly lower (139 kg/ha) than GNY in treatments R (174 kg/ha) and W (182 kg/ha) while under N0 the differences were not significant. Unlike GNC, the GNY was positively associated with mineral N supply (N_{\min}) in 0–90 cm depth in early spring ($r = 0.98$ – 0.99 and 0.83 – 0.97 for N0 and N1, resp.). Several weather and related characteristics showed relations to GNY and GNC, often opposite under N0 and N1. N_{\min} together with nitrogen fertilization rate, indicators of water regime and temperature during grain growth period explained 78–97% of observed variability of GNC and GNY in the experiment.

Keywords: water deficit; post-anthesis; grain filling; drip irrigation; precipitation; temperature; soil mineral nitrogen; winter wheat

Nitrogen concentration of wheat grain (GNC), one of the main determinants of grain nutritional value, is the result of complex processes of N uptake, assimilation and utilization. Grain protein concentration and composition, tightly associated with nitrogen nutrition, are major parameters of flour quality properties (e.g. Krejčířová et al. 2007) or barley grain quality (Váňová et al. 2006, Pettersson and Eckersten 2007). Modern high-yielding wheat cultivars require corresponding input of nitrogen to guarantee a high yield and target grain quality parameters that enable farmers to attain financial bonus. The main tools for regulating N nutrition are specific fertilization systems based chiefly on determined or estimated available supply of soil mineral N (N_{\min}), expected N mineralization and N demand by a crop (e.g. Vaněk et al. 2003, Barbottin et al. 2005).

There are distinct genotypical differences in GNC and grain quality parameters in wheat but the uptake and utilization of N are also affected by environmental factors as documented by site and year variability of the parameters (Barbottin et al. 2005, Asseng and Milroy 2006, Váňová et al. 2006, Estrada-Campuzano et al. 2008). Water availability belongs to the strongest factors determining uptake and effectiveness of N use, yield and grain quality (e.g. Haberle and Svoboda 2007, Semenov et al. 2007, Krček et al. 2008). Recently, drought in years 2000, 2001, 2003 and 2007 had a negative impact on wheat and barley production in some regions of the country. Under transition (maritime/continental) climate conditions of the Czech Republic (Tolász et al. 2007) crops are often confronted with shorter or longer periods of water shortage (Váňová et al. 2006, Trnka et al. 2007).

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Especially, the Mediterranean type of drought at critical stages of anthesis and grain filling has a detrimental effect on grain filling and quality traits (Estrada-Campuzano et al. 2008, Krček et al. 2008). Water shortage is often associated with high temperatures that are known to shorten the duration of grain growth and to reduce the yield (Wardlaw and Wrigley 1994, Triboï and Triboï-Blondel 2002). To optimize nitrogen management under adverse conditions and to minimize economical and environmental risks, better understanding of nitrogen uptake and utilization during grain growth under different levels of soil water availability is needed.

Besides common container experiments under (semi-)controlled conditions (Svobodová and Míša 2004, Ercoli et al. 2008, Krček et al. 2008) the studies of water shortage during grain formation in field experiments are often based on natural occurrence of dry years. The approach is feasible in semi-arid or Mediterranean climate but not under climate conditions of the Central Europe from where little field data are thus available. Also, the factors as different structure of canopy and root system, soil and plant nutritional and water status before anthesis complicate the interpretation and generalization of observations obtained in different years (Barbottin et al. 2005). To overcome the problem, manipulation of soil moisture in field by stationary or mobile sheltering is used (Haberle and Svoboda 2007, Estrada-Campuzano et al. 2008). The approach allows natural development of crop root system with resulting zonation of nitrogen and water uptake from a soil profile, specific microclimate conditions of the canopy and progressive advance of water shortage hardly achievable in pot experiments.

The objective of the study was to determine the effect of water deficit and ample water supply during grain filling on nitrogen concentration and nitrogen yield of winter wheat grain under field conditions.

MATERIAL AND METHODS

The experiment was carried on in the Crop Research Institute (CRI), Ruzyně in Prague, the Czech Republic (N 50°05'; W 14°20'), altitude 340 m, normal precipitation and temperature (1971–2000): 477 mm/year and 8.5°C. The field is a fertile deep loamy-clay Haplic Chernozem on loess. The soil texture (clay content < 0.01 mm is 52–56% in layers within 0–150 cm depth) and basic agrochemical

data of the experimental field are given in Svoboda and Haberle (2006). The sum of precipitation and water deficit calculated as precipitation minus reference evapotranspiration ETr (Allen et al. 1998) from March to July, main growing period of wheat, are shown in Figure 1. Comprehensive weather data from the automatic meteorological station are available at <http://www.vurv.cz/meteo/>

Three levels of water supply during grain growth period were established to investigate the effect of available water supply during grain growth period. Water shortage was induced by covering plots with mobile rain shelter during rain (treatment S), ample water supply was ensured with drip irrigation (W) and rain-fed crop served as the control treatment (R). The sheltering started between the end of stem elongation and heading, depending on soil water content and precipitation, with the aim to reach target 160–150 mm water in 0–90 cm at the start of anthesis (EC 60, Zadoks et al. 1974) and 140–150 mm at the start of grain filling (EC 70) and onward. The shelter was used only during stronger rains (> 2–3 mm) to minimize possible effect on canopy microclimate. The approach was successful thanks to accurate short-time weather forecast and on-line radar image of approaching rain clouds. Irrigation was applied to keep soil moisture at about 80% of field capacity in 0–90 cm layer. From dough stage the sheltering and irrigation were terminated. Soil water content was manipulated using data of soil sampling, calculated ETr and observed rates of wheat evapotranspiration in previous years.

There were four to six replications in R treatments constituted by 5.5 × 6 m plots, four replications in W and S treatments were performed by dividing two plots 5.5 × 8 m and two plots 5.5 × 5 m, resp., to sub-plots. The cultivar of winter wheat (*Triticum aestivum* L.) Nela used in the experiment has been widely grown in the Czech Republic, it belongs to quality group A (high quality) and it has a good spring regeneration, tillering, plasticity and yield stability, plants are about 85 cm high.

Wheat was grown without nitrogen and with N rate 200 kg/ha (N0 and N1, resp.). Mineral N ($N_{\min} = N\text{-NO}_3^- + N\text{-NH}_4^+$) and soil moisture in soil layers to the depth of 90–130 cm in 20 cm increments were determined during the growth (Haberle and Svoboda 2007). Grain N concentration (GNC) was determined on Elementar Analyser EuroEA 3028-HT (EuroVector, Milan, Italy) and SAN^{PLUS} System (SKALAR) in four replications, the grain N yield was calculated from GNC and grain yield.

Statistical evaluation. The effects of year, water status and N fertilization treatments on GNC and GNY were examined with two- and three-way analysis of variance (ANOVA), the differences between treatment means were tested with Tukey test at $P < 0.05$. The relations between GNC or GNY and water and N_{\min} supply, temperature or grain growth duration were examined with linear regression analysis. Statistical software UNISTAT was used.

RESULTS AND DISCUSSION

The results of four-year field experiment with winter wheat aimed at the effect of post-anthesis water supply on grain nitrogen concentration and grain N yield are presented.

Weather and water supply in experimental years

Weather conditions during the main growth period of winter wheat are shown in Figure 1. The sums of precipitation from March (January) to the end of July were 256 (312) mm, 322 (380) mm, 315 (336) mm and 264 (318) mm in respective years, i.e. about long-term normal 289 (358) mm, but with great variability among months (Figure 1). Except for 2004, average temperatures in April, June and July were above normal, April, May and June 2007 and July 2006 significantly increased above normal temperatures (Kožnarová and Klabzuba 2002). The year 2006 had the highest sum of average and maximum temperatures above 22°C and 30°C respectively, from anthesis to the end of grain filling.

Using rain shelter (treatment S) and drip irrigation (W) soil water content was managed to simulate the effect of dry and wet growth seasons during grain formation. In treatment S water content in 0–90 cm zone was reduced from initial 200 mm, 245 mm, 260 mm and 155 mm at the end of stem elongation or booting in respective years 2004–2007 to 150–170 mm and 140–150 mm water at heading and at the start of grain filling, respectively. Soil moisture dropped to the low levels in drought periods of years 1995, 1997, 1998, 2000 and 2003 under winter wheat in the same experimental field but for shorter period than maintained in this experiment (Haberle et al. 2002, and unpublished). Permanent wilting point (pF 4.2) is 100–130 mm in the layers of 0–90 cm zone, however, neither in this experiment nor in previous years terminal drought of plants was observed. The roots of winter wheat reached under 90 cm depth to about 110–130 cm in the experimental field (Svoboda and Haberle 2006) and they were able, despite a low density, to extract some water and nitrogen from the deep subsoil zone (Haberle et al. 2006). In treatment W by 80–100 mm higher water content than in stress treatment was maintained from anthesis. In rain-fed crop (R) initially high moisture level in 2004–2006 was gradually depleted during grain filling and ripening in 2004 and 2006, while in 2005 the supply was replenished during July. In 2007 exceptionally low water content during vegetative growth was followed by a slow increase to levels observed in 2004 and 2006.

Grain N concentration

The concentration of grain N (GNC) ranged between 1.30% and 1.86% in N0 and between

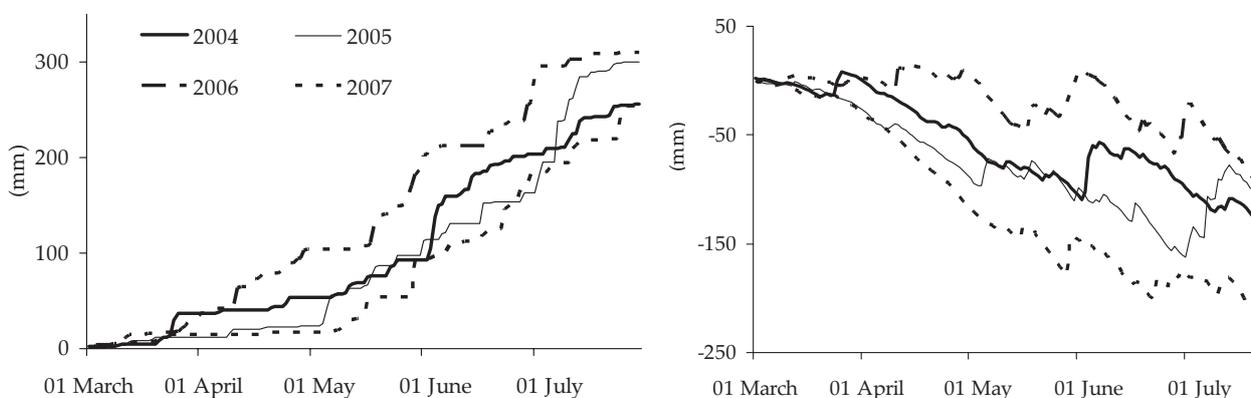


Figure 1. Cumulated precipitation (left) and calculated water deficit (right) from March to July in Prague-Ruzyně

Table 1. The effect of water and nitrogen treatments, and year on the nitrogen concentration and nitrogen yield of wheat grain

		Grain nitrogen concentration (%)	Grain nitrogen yield (kg/ha)
Nitrogen	N0	1.54 ^a	92 ^a
	N1	2.27 ^b	165 ^b
Year	2004	1.88 ^a	179 ^a
	2005	1.70 ^a	118 ^b
	2006	1.92 ^a	121 ^b
	2007	2.13 ^a	96 ^b
Water	treatment R	1.89 ^a	133 ^a
	treatment S	2.01 ^a	110 ^a
	treatment W	1.81 ^a	142 ^a
Significance (ANOVA)			
N		***	***
Year		***	***
Water		***	***
Water × N		***	*
Water × year		**	NS
N × year		***	***
Water × N × year		NS	NS

Means within the groups (water, year, nitrogen) followed by the same letter are not significantly different ($P < 0.05$, Tukey test); NS – not significant; *, **, ***significant at the 0.05, 0.01 and 0.001 probability levels

1.70% and 2.87% in N1 in experimental years. Average GNCs in treatments S, R and W were 1.52, 1.54 and 1.56% in N0 and 2.50, 2.24 and 2.07% in N1, respectively (Tables 1 and 2). The effects of water supply, year and nitrogen treatments on GNC were highly significant ($P < 0.001$) as well as the interaction of water and nitrogen. When the analysis was performed separately for N fertilization treatments (Table 2) the effect of water treatment on GNC was highly significant in N1 ($P < 0.001$) but not significant in N0 ($P = 0.23$). Water stress significantly ($P < 0.05$) increased GNC in comparison with irrigated and rain-fed fertilized wheat (Table 2).

Weather conditions and duration of grain filling

Several characteristics of weather conditions during vegetative growth and period of grain development showed relation with GNC, often opposite under N0 and N1 fertilization treatments.

Monthly temperatures in April, May and June (not July), and average reference evapotranspiration (ET_r) were mostly in significant positive relation to GNC in N1 and negative in N0. Similar but weaker correlations were observed for sum of daily average temperatures above 22°C and daily maximum temperature above 30°C during grain development. The optimum average temperature during grain growth is generally considered to be less than 20–25°C for wheat, daily maximum temperatures above 30°C were found to reduce the yield of wheat (e.g. Wardlaw and Wrigley 1994, Triboï and Triboï-Blondel 2002, Barbottin et al. 2005). The relations between GNC and precipitation, average soil water content and apparent water use during grain growth in experimental years were mostly negative and inconsistent in our experiment. In accordance with the above findings there was a significant negative relation between GNC and duration of grain development calculated from heading or from anthesis to dough stage or maturity (correlation coefficient $r = -0.89$ to -0.93 for the water treatments) in N1,

Table 2. The effect of water treatments and year on grain nitrogen concentration and grain nitrogen yield under N0 and N1 fertilization

	Treatment	Grain nitrogen concentration		Grain nitrogen yield	
		N0	N1	N0	N1
Water	R	1.54 ^a	2.24 ^{a, b}	93 ^a	174 ^a
	S	1.52 ^a	2.50 ^a	81 ^a	139 ^b
	W	1.56 ^a	2.07 ^b	102 ^a	182 ^a
2004	R	1.79 ^{a, b}	1.94 ^a	157 ^{a, b}	209 ^a
	S	1.86 ^a	2.35 ^b	142 ^a	167 ^b
	W	1.64 ^b	1.70 ^a	183 ^b	214 ^a
2005	R	1.30 ^a	1.98 ^a	74 ^a	172 ^a
	S	1.37 ^{a, b}	2.22 ^a	62 ^a	140 ^a
	W	1.48 ^b	1.86 ^a	88 ^a	174 ^a
2006	R	1.49 ^a	2.26 ^a	78 ^a	182 ^a
	S	1.45 ^a	2.57 ^a	71 ^a	138 ^a
	W	1.49 ^a	2.24 ^a	74 ^a	181 ^a
2007	R	1.58 ^a	2.80 ^{a, b}	61 ^a	132 ^{a, b}
	S	1.42 ^b	2.87 ^a	47 ^b	111 ^a
	W	1.61 ^a	2.48 ^b	63 ^a	159 ^b
Significance (ANOVA)					
Year		***	***	***	***
Water		NS	***	***	***
Water × year		***	NS	NS	NS

Means in the same group (water, years) followed by the same letter are not significantly different ($P < 0.05$, Tukey test); NS – not significant; *, **, ***significant at the 0.05, 0.01 and 0.001 probability levels

and not-significant for treatment R calculated from anthesis ($r = -0.67$ and -0.56). No such consistent association between GNC and duration of grain growth was found in N0.

The observed effects of water treatments on grain N concentration are in general agreement with previously published results (Wardlaw and Wrigley 1994, Triboï and Triboï-Blondel 2002, Asseng and Milroy 2006, Martre et al. 2006). Both pot and field experiments described in literature showed increased grain nitrogen concentration or protein content under water shortage and/or high temperatures. It is explained by the fact that the loading of N into grains is not as sensitive to the factors as carbon filling. A weak effect of water shortage on GNC under nitrogen deficiency described Ercoli et al. (2008) and others. Triboï and Triboï-Blondel (2002), Martre et al. (2003, 2006), Triboï et al. (2006) and Semenov et al. (2007) proposed and verified conceptual model based on

source-sink relationships that explains the interacting effects of water and nitrogen supply, low and high temperature during post-anthesis period on N (protein) concentration and yield in wheat. In summary, carbon and nitrogen metabolism are relatively independent. However priority is given to nitrogen metabolism in grains (Egle et al. 2008) which regulates the duration of grain growth and also nitrogen uptake by roots and senescence and therefore the duration of carbon assimilate production (Triboï and Triboï-Blondel 2002).

Soil N_{\min} supply at tillering and heading

GNC only loosely ($|r| = 0.41-0.72$) correlated with N_{\min} content (0–90 cm) in spring, positively in N0 and negatively in N1 (Figure 2). N_{\min} supply before the start of flowering (sampling about heading) had no consistent effect on GNC. Under

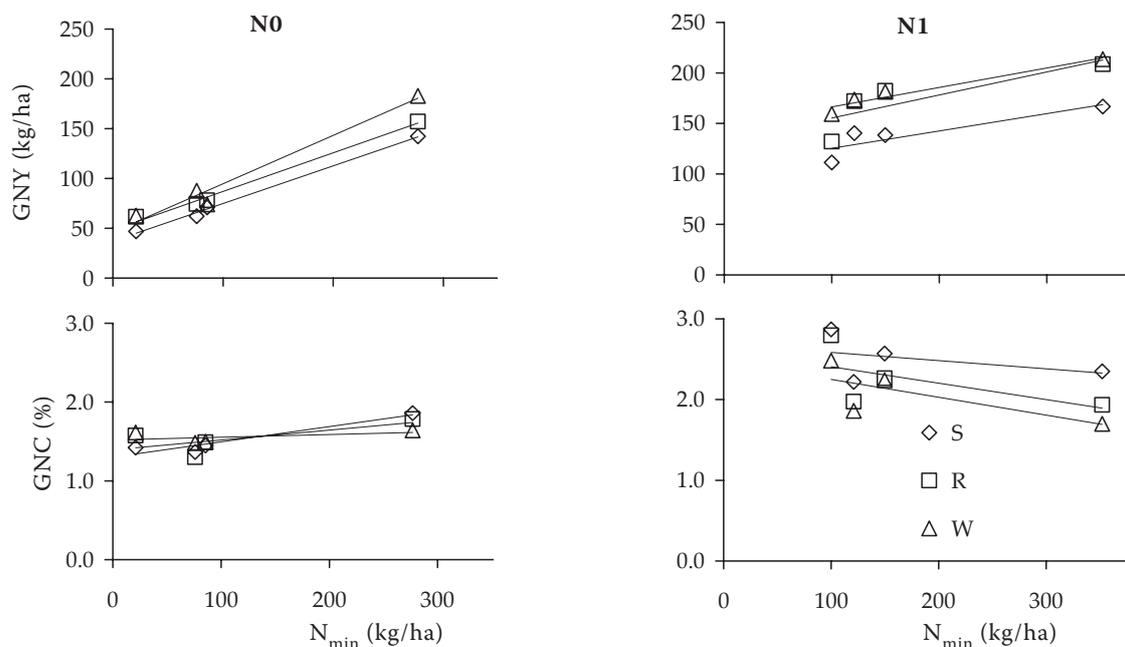


Figure 2. The relation between soil mineral N content in spring (N_{\min} , 0–90 cm) and grain N yield (GNY) and grain N concentration (GNC) under water shortage (S), rain-fed conditions (R) and irrigation (W) during grain growth. Linear regression lines for the water treatments are shown

N0 the mineral N was very low before flowering (4–19 kg/ha), except for the year 2004 (12–36 kg/ha). In the treatment N1 the N_{\min} supply was about 50 kg N/ha, in year 2004 about 200 kg N/ha. Our results show that spring N_{\min} content was not reliable predictor of GNC variability among years without considering other factors. For example, in 2004 GNC in N1 was lower than in other years while the N_{\min} supply was by more than 100% higher. Similarly, Pettersson and Eckersten (2007) did not find a significant correlation between N_{\min} at sowing (0–60 cm) and grain protein concentration of barley in 16 fertilization trials in Sweden, unlike the day of sowing probably connected with the risk of high temperatures during grain filling. Of course, there is a positive association between increasing N fertilizer rates and GNC within the same environment (year, site) and genotypes.

As for other factors possibly affecting N uptake, the proportion of ammonium nitrogen was low throughout the experiment hence no significant interaction with water or temperature variability could be expected (Trčková et al. 2006). Also, year-to-year differences of root growth (Svoboda and Haberle 2006, Herrera et al. 2007) may influence N uptake from deep subsoil layers during grain filling. However, the concentration of N_{\min} under 90 cm was low (1–5 mg/1000 g soil), in the experiment except for the year 2004 (about 10 mg/1000 g soil). Also, the treatments and years did not substantially alter root depth (not published).

Taking into consideration opposite effect of temperature and water under high and low nitrogen supply in our experiment multiple regression analysis was performed separately for N0 and N1. N_{\min} in spring, average temperature in May and June and relative water input (rain + irrigation) during main growth period (S/R, W/R, R = 1.0) explained 89% (N0) and 80% (N1) of GNC variability throughout the experiment. When predicted GNC of treatments N0 and N1 were compared with observed GNC the fit was good (Figure 3).

Grain nitrogen yield

Water regime, nitrogen fertilization and year had a significant effect ($P < 0.01$) on grain nitrogen yield (GNY) (Table 1). When GNY was analysed separately for N0 and N1 water and year effects were also significant ($P < 0.01$), the interaction between water and year was not significant.

The effect of water regime on GNY was opposite to GNC, water stress decreased and ample water supply increased GNY. On average, N yield of grain in N1 was 139 kg, 174 kg and 182 kg N/ha in treatments S, R and W, respectively. Respective values in N0 were 81 kg, 93 kg and 102 kg N/ha. GNY of fertilized wheat under stress was significantly lower ($P < 0.05$) than GNY in R and W, while under N0 the differences were not significant. Water stress reduced GNY by 21% in N0 and by 23% in

N1, in comparison with ample water supply, in individual years the reduction ranged between 19–30%, except for N0/2006 (4%). The decrease of GNY under post-anthesis drought is the result of lesser demand for N and reduced uptake of soil N (Martre et al. 2003, Asseng and Milroy 2006, Haberle and Svoboda 2007). Substantial amounts of N, more than 60%, may be depleted after anthesis in some years depending on crop demand for N and growing conditions (Semenov et al. 2007, Egle et al. 2008). In our experiment N_{\min} supply before anthesis was higher in stressed wheat (not shown) in contradiction with lower N uptake, but it is not possible to distinguish whether impaired N availability due to dry soil or reduced demand for N was the most important.

Weather conditions

Average month temperatures from March (N1) or April (N0) to June and ETr showed consistently negative mostly significant correlation with GNY. However, the interpretation of the observations should be tentative as there was coincidence of the highest N_{\min} supply and GNY with coldest year (2004) and *vice versa* (year 2007). Unlike GNC, duration of grain growth was only loosely (except for S) related to GNY. The effects of precipitation or average soil water content during grain development were weak.

N_{\min} supply at tillering and heading

On the contrary to GNC, the GNY was tightly associated with N_{\min} supply (0–90 cm) in spring in

N0 ($r > 0.98$) and in N1 ($r = 0.83–0.97$) (Figure 2). The effect of N_{\min} content before flowering on GNY was positive but weak. Introducing average temperature in May and June and relative water input (or dummy variables for water treatments) with spring N_{\min} into multiple linear regression explained 97% (N0) and 78% (N1) of GNY variability observed in the experiment. Similarly to GNC when predicted GNY was compared with observed GNY the fit was good (Figure 3).

The impact of post-anthesis water regime or temperature on grain N concentration and N yield is determined by the effect of the factors on uptake and assimilation of N and also by the influence on the mobilization of previously assimilated N and C from vegetative tissues (Barbottin et al. 2005, Egle et al. 2008, Ercoli et al. 2008). We observed a consistently lower N concentration in straw at harvest as the result of post-anthesis water stress, by 0.09% (N0) and 0.16% (N1), in comparison with irrigated treatment that suggests higher remobilization of N or lower one of C. The effects of water regime on assimilation and remobilization of C and N during grain filling (Barbottin et al. 2005, Triboi et al. 2006) were reflected in C and N yields – GNY reduction due to stress was higher (21%) than average reduction of grain yield (15%) under N0 while under N1 the reduction of GNY (23%) was lower than yield reduction (33%).

It should be stressed that the presented results were obtained with one cultivar. The generalization is hardly feasible as genotypically based specific responses to high temperature, water stress and available nitrogen were proved in cereals (e.g. Asseng and Milroy 2006, Triboi et al. 2006, Estrada-Campuzano et al. 2008). On the other side, it may be expected that the impact of

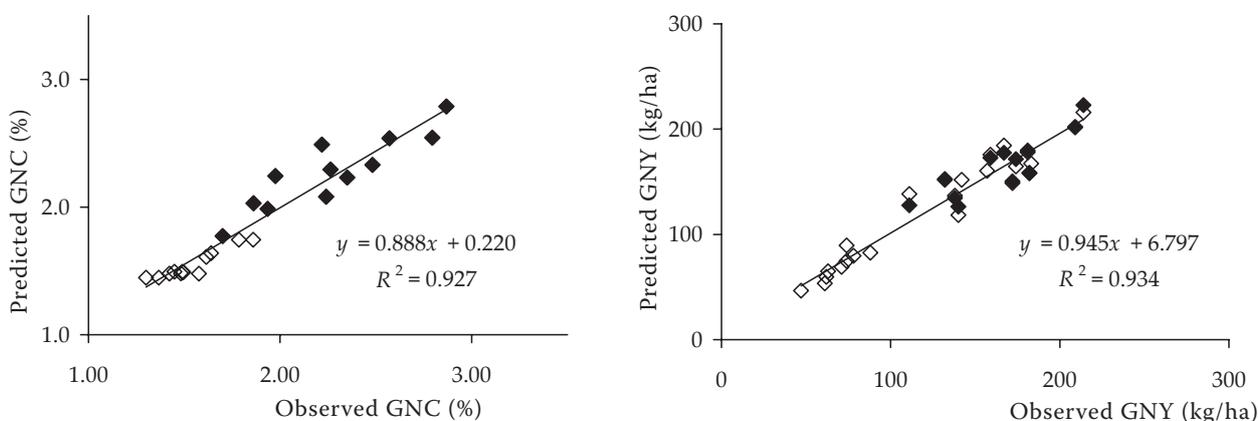


Figure 3. The relation between observed and predicted GNC and GNY. Spring N_{\min} (0–90 cm), average temperature in May and June and relative water input (see text) were used in multiple linear regression analysis calculated for N0 (open symbols) and N1 (closed symbols) data

markedly different water regimes during grain growth will be similar at least within a group of wheat cultivars used by farmers under the same specific soil-climate conditions.

The main results of presented experiment may be summarized as follows. The post-anthesis water regime significantly and consistently affected grain nitrogen concentration and grain N yield of winter wheat. The N_{\min} supply in spring, at the start of main growing period, together with N rate and indicators of water regime and temperature during grain growth period explained 78–97% of observed variability of GNC and GNY among years and treatments in the experiment. The findings contribute to the estimation of N demands by wheat crop and to interpretation and early predictions of the impact of different weather conditions on final grain quality of winter wheat.

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