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The effect of different post-anthesis water supply on the carbon isotope discrimination of winter wheat grain

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ABSTRACT

Isotopic carbon discrimination ($\Delta^{13}C$) of winter wheat grain grown under different water and nitrogen supplies was determined. In two field experiments during years 2004–2007 (A) and 2008–2013 (B), a water shortage was induced from the flowering stage on with a mobile shelter (S), while an optimal water supply was ensured with drip irrigation (I), and a rain-fed crop served as the control treatment (R). Water supply had a statistically significant effect on grain $\Delta^{13}C$ values in both experiments ($P < 0.01$). The average values of grain $\Delta^{13}C$ in treatments I, R and S were 19.43, 18.68 and 17.70‰ (A); and 20.36, 19.60 and 18.13‰ (B). Grain $\Delta^{13}C$ was in a significant linear relationship ($P < 0.01$) with the amount of water supplied by precipitation or irrigation. The regressions suggested that grain $\Delta^{13}C$ increased by 1.14‰ and 1.16‰ (A), and 0.98‰ or 0.96‰ (B) for every 100 mm of water from January and March, respectively, until the early dough stage ($r = 0.79–0.74$, $P < 0.05$). Pooled data for the whole period 2004–2013 showed increases of 1.06‰ and 1.08‰ ($r = 0.91$ and 0.82, $P < 0.05$) for 100 mm of water, respectively. The results of the experiment confirmed the stable and predictable effect of water supply on wheat grain $\Delta^{13}C$.

Keywords: *Triticum aestivum* L.; rainfall; evapotranspiration; water stress; nitrogen fertilization

The available supplies of water and nitrogen determine to a great extent the growth, yield, and quality of field crops. Fluctuations of precipitation during dry spells and increasing evapotranspiration due to higher temperatures result in the frequent occurrence of periods of water shortage during the growth of crops; and climate change will probably only worsen this situation (Trnka et al. 2014).

Under the transition (maritime/continental) climatic conditions of the Czech Republic, water shortages in annual crops mostly occur during the main growth period, when the winter supply of soil water is exhausted and precipitation is not sufficient to cover the transpiration demand. In cereals, water shortage at critical stages of anthesis and grain growth has a negative effect on grain filling and quality traits (Guttieri et al. 2001, Balla et al. 2011, Dai et al. 2016).

In the process of photosynthesis, C3 species discriminate between the heavier isotope ^{13}C and the lighter ^{12}C. Under a water shortage, plants close their stomata to reduce water consumption through transpiration, in turn leading to different concentrations of CO_2 inside the leaves, and to changes in the ^{13}C and ^{12}C isotopic ratio (Farquhar et al. 1989). The rate of CO_2 concentration inside the leaves and in the atmosphere correlates with the values of $\delta^{13}C$. The value of δ^{13} is calculated as the rate of the isotopes in a sample and in a standard. For the description of changes in C isotopes, the following value for ^{13}C discrimination is used:

$$\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p(1000))$$

Where: $\delta^{13}C_a$ – $\delta^{13}C$ value of air (–8‰); $\delta^{13}C_p$ – measured value of the plant (Condon et al. 1987).

Those plants (genotypes) that are able to sustain their stomata open for longer periods of time under water shortage conditions have a higher $\Delta^{13}C$, which suggests higher photosynthetic activity as a precondition for attaining a higher yield. As a result, a correlation between yield and grain $\Delta^{13}C$

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was observed, especially when plants that were either under strong stress (semiarid conditions) or under irrigation were compared (Zhu et al. 2009, Misra et al. 2010). The value of $\Delta^{13}C$ in seeds integrates the effects of water supply and plant water status during growth. However, this assumption about a positive correlation between yield and $\Delta^{13}C$ has not always been confirmed, as the response to water shortage and the process of yield formation depends on many year-, agronomy-, and site-dependent factors during growth (Monneveux et al. 2005). Under temperate and transition (maritime/inland) climatic conditions, where precipitation during growth is highly variable, rain-out shelters enable a standardization of the water supply in the field, partially independent of that year's meteorological conditions (e.g., Yasir et al. 2013).

The aim of the study was to examine the impact of water supply differentiated from anthesis, during the period of wheat grain growth upon values of $\Delta^{13}C$, to determine the relationships with water supply during growth and any possible interactive effect of nitrogen fertilization.

MATERIAL AND METHODS

Two successive experiments (A and B) with differentiated water supplies during grain growth were carried out at the Crop Research Institute (CRI), Ruzyně near Prague, Czech Republic (50°05'N, 14°20'W), altitude 340 m a.s.l., average annual sum of precipitation 477 mm, and average daily temperature (1971–2000) of 8.5°C. The experimental field is a fertile deep loamy-clay Haplic Chernozem soil on loess. The contents of available P, K, and Mg (according to Mehlich III) were 85, 245 and 226 mg/kg dry soil in the topsoil (0–30 cm); and 10–12, 121–141 and 254–352 mg/kg in the subsoil layers (30–120 cm). Soil pH is 7.0–7.3 and humus content 2.5% (topsoil). Maximum soil moisture of the top and subsoil layers, mostly observed in the spring (34–38.5 vol. %), corresponded to lower values of laboratory-determined water tension at pF 2.0 (37.3–46.1 vol. %). Detailed data on the experimental field, as well as the description of manipulation of water content are given in Haberle et al. (2008), and Raimanová and Haberle (2010). The crop rotation was (1) cereal fodder-pea mixture; (2) winter oilseed rape, and (3) wheat (experimental). Phosphorus and potassium were applied in mineral fertilizers before winter wheat in the rates 33 kg P/ha and 84 kg K/ha. Accumulated daily precipitation and water deficit, calculated as the precipitation minus the reference evapotranspiration (Allen et al. 1998) from March to July in the experimental years are shown in Figure 1. Comprehensive weather data are available at http://www.vurv.cz/meteo/.

Water treatments. Three levels of water supply during the grain growth period were established. Water shortage was induced by the covering of plots with a mobile rain-out shelter, with roof and sideboards from clear acrylic plastic, during rain events (treatment S), an ample water supply was ensured with drip irrigation (I), and a rain-fed crop served as the control treatment (R). The sheltering usually started before anthesis, depending on soil water content and precipitation, with the aim of reaching 150–160 mm water in 0–90 cm at the start of anthesis, and 140–150 mm during grain filling. The permanent wilting point (pF 4.2) is 110–130 mm in the layers of the 0–120 cm zone; permanent wilting of wheat plants was neither observed during the experiments nor in previous years. The shelter was only used when stronger rains (approximately > 3 mm/day) were expected. Irrigation was applied to keep the soil moisture above 60% of field capacity in the 0–90 cm layer during grain filling. Irrigation was stopped about the early dough stage to reduce the risk of lodging and grain pathogens. Soil water content was manipulated using data from the soil sampling, calculated reference (Allen et al. 1998), and observed rates of wheat evapotranspiration, as well as water depletion from the root zone in the field in previous years (Haberle et al. 2008, Raimanová and Haberle 2010).

In the split-plot design there were four replications in the R N1 and N2 treatments consisting of 33 m^2 plots; four replications in the I and S treatments were performed by dividing the area of two plots of 44 m^2 (I), and two plots of 27.5 m^2 (S), respectively, into sub-plots during the years 2004–2007 (A). During 2008–2012 (B), two cultivars were sheltered and irrigated; thus, one half of the respective areas was used.

Winter wheat (*Triticum aestivum* L.) cv. Nela was grown in years 2004–2007 (experiment A), cvs. Anduril and Biscay in years 2009–2011, cvs. Bagou and Manager in year 2012 and 2013 (experiment B). For the study, average data from the two cultivars were used. The wheat was grown without nitrogen (N1),

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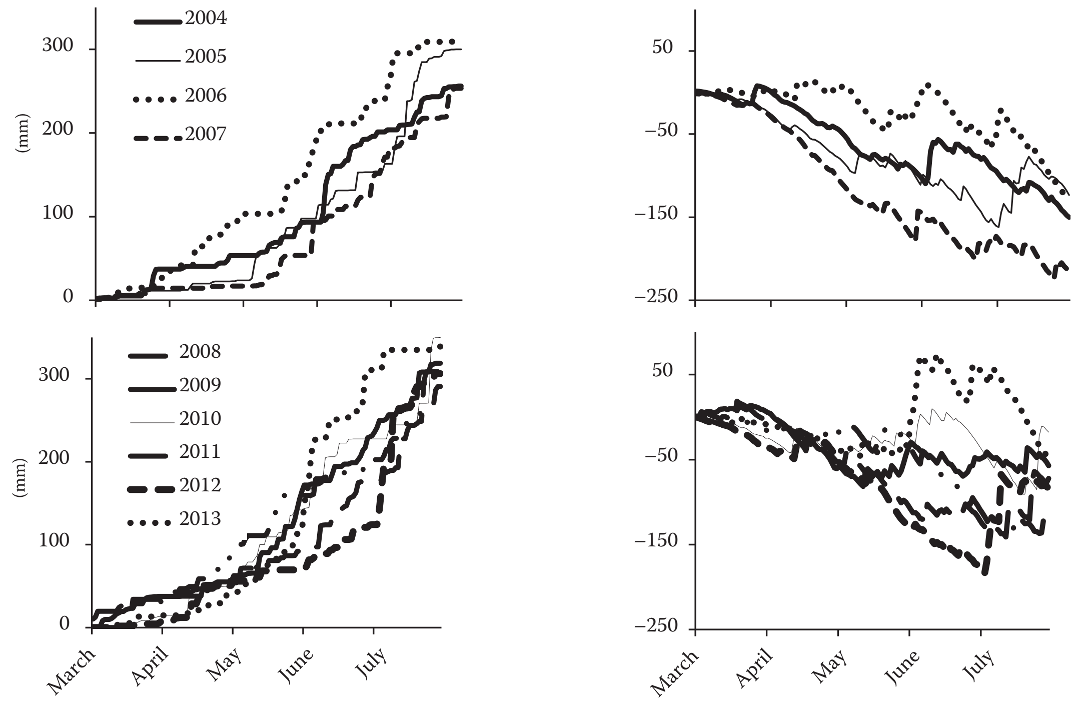

Figure 1. Accumulated precipitation and water balance (precipitation minus reference evapotranspiration) from 1st March in years 2004–2007 and 2008–2012

and with 200 kg N/ha (N2) during years 2004–2007 (A); and with 0–30 kg N/ha (N1) and 140–180 kg N/ha (N2), according to spring soil mineral N supply, during 2008–2012 (B). Both the soil moisture and mineral nitrogen (N_{min}) in the top 10 cm, as well as to a depth of 130 cm in 20 cm increments, were determined during growth. The water volume was calculated from the gravimetric soil moisture and soil bulk density. Ears were sampled during growth and at maturity. Fifteen ears per a plot were sampled at maturity. The grains were hand separated, dried, homogenized into a fine powder in a MM301 ball mill (Retsch, Haan, Germany). The content of carbon (C) isotopes in the grains was determined in 4 replications with an EA 3200 elemental analyzer (Eurovector, Redavalle, Italy) connected with an Isoprime isotope mass spectrometer (GV Instruments, Wythenshawe, UK) in the Crop Research Institute's laboratory. The values of $\delta^{13}C$ and $\Delta^{13}C$ were calculated.

The effects of water regime, nitrogen (N) fertilization, and year on $\Delta^{13}C$ were examined using three-way ANOVA; according to the Kolmogorov-Smirnov test, the data had a normal distribution. The differences between treatment means were tested with the Tukey's *HSD* test, linear regression analysis was performed. Statistica CZ 12 software (StatSoft, Inc., Tulsa, USA) was used.

RESULTS AND DISCUSSION

The differentiated water supply after flowering. The differentiated water supply after flowering had a significant effect ($P < 0.01$) on grain $\Delta^{13}C$ values of winter wheat at maturity in both experiments (Table 1). In agreement with the theory and published experimental data, the shortage of water reduced grain $\Delta^{13}C$. The average values of grain $\Delta^{13}C$ in treatments I, R and S were 19.43, 18.68 and 17.70‰ (exp. A); 20.35, 19.62 and 18.10‰ (exp. B). The differences among the water treatments were significant ($P < 0.05$) in both experiments (Table 1). According to the analysis of variance, the water supply and year explained 49.4% and 41.7% (A), and 51.3% and 18.6% (B) of the total variability of grain $\Delta^{13}C$ data. The optimal water supply provided by irrigation, on average, increased grain $\Delta^{13}C$ by 0.75‰ and 1.73‰ (A), and by 0.73‰ and 2.25‰ (B), when compared with the rain-fed and stressed wheat, respectively.

The values of $\Delta^{13}C$ in our experiments were higher than those observed in wheat from drier environments. The lowest values of $\Delta^{13}C$ observed in stress treatment during the dry years 2007 and 2012 (16.0‰ and 16.9‰) were at the upper level of the data ($\Delta^{13}C < 15.25$‰) observed by Dalal et

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Table 1. The results of three-way ANOVA

Factor	P	Factor	Experiment A	P	Factor	Experiment B
		R	18.68[b]		R	19.62[b]
Water	< 0.01	I	19.43[c]	< 0.01	I	20.35[c]
		S	17.70[a]		S	18.10[a]
Nitrogen (N)	< 0.01	N1	18.88[b]	< 0.01	N1	19.48[b]
		N2	18.50[a]		N2	19.16[a]
		2004	18.65[b]		2008	19.91[d]
		2005	19.27[c]		2009	19.29[c]
Year	< 0.01	2006	19.10[c]	< 0.01	2010	18.83[ab]
		2007	17.74[a]		2011	19.12[bc]
		–	–		2012	18.45[a]
		–	–		2013	20.39[e]
		R/N1	18.96[c]		R/N1	19.48[c]
		R/N2	18.43[c]		R/N2	19.51[c]
Water × N	< 0.01	I/N1	19.37[d]	0.041	I/N1	20.42[d]
		I/N2	19.49[d]		I/N2	20.29[d]
		S/N1	18.18[b]		S/N1	18.37[b]
		S/N2	17.21[a]		S/N2	17.89[a]
Water × year	< 0.01			< 0.01		
N × year	< 0.01			0.820		
Water × N × year	< 0.01			0.694		

Significant differences (at < 0.05) between average values of grain $\Delta^{13}C$ are shown with different letters. R – control; S – water shortage; I – irrigation; N1 – low N fertilization; N2 – high N fertilization

al. (2013) in a semi-arid environment. The data of Wang et al. (2013), 16.3–18‰; Zhu et al. (2009), 16–19‰; Clay et al. (2001) 14–19‰; Yasir et al. (2013) 16.14–19.05‰ or Monneveux et al. (2005) 14.5–19‰ corresponded to our experimental data – with the lowest values attributed to stressed wheats in the published experiments. In most of these field experiments, water stress was affecting plants from the earlier stages of development with corresponding stronger depression of grain yields. In our experiments, on average, water shortage after flowering reduced the yields by 12.29% (N1) and by 19.78% (N2) when compared with the rain-fed treatment. Average grains yields in years 2004–2013 reached 7.25, 6.76 and 5.92 t/ha (N1) and 8.61, 7.91 and 6.36 t/ha (N2) in treatments I, R and S, respectively. The coefficient of variation of grain yields ranged from 12.2% (N2/I) to 24.1% (N1/I).

The differentiated water supply after flowering. The differentiated water supply after flowering was significant ($P < 0.01$) and the means of N1 and N2 were significantly different in both experiments. Nitrogen fertilization interacted with the water supply but in experiment B the interaction was weaker (Table 1). Nitrogen fertilization decreased grain $\Delta^{13}C$, on average, by 0.97‰ and 0.53‰ under stress and rain-fed conditions and increased it by 0.12‰ in the irrigated wheat in experiment A. In experiment B, the S and I treatments reduced grain $\Delta^{13}C$ by 0.48‰ and 0.13‰, while under rain-fed conditions the effect was negligible (+0.03‰) (Table 1). The effect of N fertilization was modified by N_{min} supply. For example, in 2004 fertilization reduced $\Delta^{13}C$ only under stress (by 0.20‰) probably due to an exceptionally high content of soil N_{min} in spring 2004 after the dry year 2003. Similar interactions between the effect of water availability and nitrogen supply on $\Delta^{13}C$ were described by Clay et al. (2001), Wang et al. (2013), Dalal et al. (2013), as well as others. The effect of N fertilization may be attributed to the faster depletion of water by a more vigorous crop stand with a higher number of stalks and greater leaf area, as also observed in this experiment (results not shown). Wang et al. (2013) observed that the water supply had positive

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effects, while a higher N supply had negative effects on the gas exchange parameters; further, that it resulted in negative relationships of $\Delta^{13}C$ to N contents in the grain, similarly to our observations (not presented). In agreement with our results, N fertilization reduced grain $\Delta^{13}C$ from 18.0–16.8‰ in their experiment. On the other side, higher (apparent) utilisation of N under conditions of a good water supply and the increased efficiency of grain nitrogen use are positively correlated with the grain $\Delta^{13}C$ of wheat (Dalal et al. 2013), possibly due to increased stomatal conductance (the inverse to conditions of less water) as well as larger photosynthetic capacity of the wheat plant (Condon et al. 1987, Clay et al. 2001).

The differentiated water supply after flowering. The differentiated water supply after flowering was significant ($P < 0.01$) in both experiments in spite of the same water supply treatments during grain growth. As a significant part of grain carbon comes from the reutilization and redistribution of C-substances produced during pre-anthesis growth (e.g., Man et al. 2015), the effect was probably caused by the differential availability of water (precipitation, soil supply) before grain formation, resulting in different ^{13}C discrimination. It was confirmed by the low grain $\Delta^{13}C$ of all treatments (16–18.7‰) in exceptionally dry year 2007. Also, some proportion of the water is absorbed by plants from under the target 90 cm depth. We observed the roots grew deeper and apparently deplete the nitrogen and water from under the depth of 90 cm (Haberle and Svoboda 2014).

Grain $\Delta^{13}C$ was in a significant linear relationship ($P < 0.05$) with the amount of water supplied by precipitation and irrigation. Almost the same correlations were found between $\Delta^{13}C$ and the sum of precipitation from January or March 1st to the early dough stage (about DC 80) (Figure 2). A less tight correlation was found when precipitation from sowing was used. Additionally, using precipitation sums to maturity weakened the relationships, due to the occasional occurrence of strong rains during the last period of ripening (i.e., in the phase when photosynthesis and formation of substances possibly displaying ^{13}C discrimination cease). The regressions suggest that wheat grain $\Delta^{13}C$ increased by 1.14‰ and 1.16‰ (A), and 0.98‰ and 0.96‰ (B) for every 100 mm of water from January or March to the early dough stage (r = 0.79–0.74, $P < 0.05$,

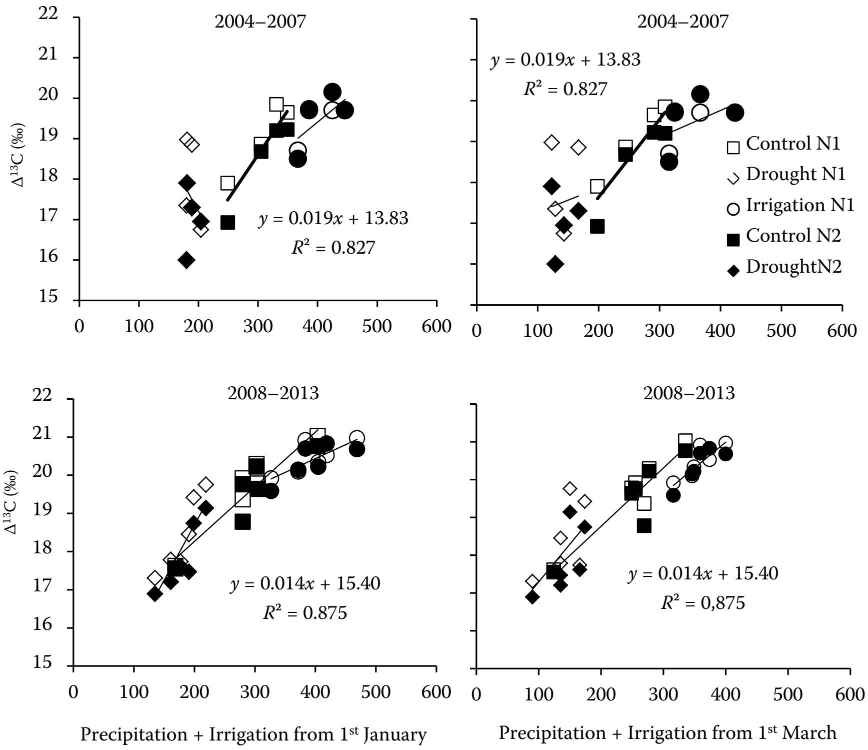

Figure 2. The relationship between the supply of water from precipitation and irrigation summed from 1st January or 1st March to early dough stage of development and values of grain $\Delta^{13}C$ in 2004–2007 and in 2008–2013. The regressions for the pooled data of the control treatment are shown. N1 – low N fertilization; N2 – high N fertilization

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N = 24 and N = 36 in A and B experiments) (Figure 1). Pooled data for the whole period 2004–2013 showed an increase by 1.06‰ and 1.08‰ for 100 mm of water between 90 mm and 425 mm (r = 0.91 and 0.82, P < 0.05, N = 60) for 100 mm of water, respectively. In comparison, under semi-arid conditions (Dalal et al. 2013), wheat grain $\Delta^{13}C$ between 13.5–16.5‰ was observed under only 11–76 mm precipitation from sowing to anthesis (plus available water supply in 0–150 cm) or 97–189 mm precipitation (from sowing to harvest) and under a high evaporative demand.

The results of two field experiments with water supply differentiated during grain growth proved a systematic modification of wheat grain $\Delta^{13}C$ by the supply of water. The quantified robust relationships between grain $\Delta^{13}C$ and total water supply may be used as an indirect indicator of water availability during grain growth. The analysis of wheat grain $\Delta^{13}C$ proved to be sensitive enough to differentiate between crops with different growth due to different N fertilization and soil supply, and may be used as a basis for comparison of water shortages on wheat cultivars.

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