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YIELD FORMATION, QUALITATIVE PARAMETERS OF WINTER WHEAT GRAIN AND CROP DAMAGE DEPENDING ON METHOD OF NITROGEN FERTILIZER APPLICATION ("CONTROLLED UPTAKE LONG TERM AMMONIUM NUTRITION" OR SPLIT APPLICATION)

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ABSTRACT

The influence of nitrogen injection on winter wheat (*Triticum aestivum* L.) yield, yield formation, stem lodging, fungal diseases incidence and qualitative parameters of grain was studied. The injection of all nitrogen applied in one dose, so-called CULTAN (Controlled Uptake Long Term Ammonium Nutrition) system, was compared with nitrogen split application during the years 2009-2013 in small-plot field experiments at three experimental sites in the Czech Republic (central Europe). Nitrogen dose applied conventionally on soil surface was split into three partial applications. The CULTAN fertilization was carried out at the beginning of spring vegetation instead of usual term, i.e. the end of tillering (BBCH 29). Grain protein content was significantly lower and grain starch content significantly higher in wheat treated with the CULTAN system compared to the nitrogen split application. Nevertheless, the CULTAN treatment did not lead to significantly higher starch yield in comparison with the nitrogen split application. Plants per m^2, number of ears per m^2 and thousand grain weight were not significantly different among nitrogen treatments. Significantly lower number of grains per ear was recorded while using the CULTAN system compared to the nitrogen split application when 150kg N/ha was applied, but this had no significant impact on grain yield. No positive effect of the CULTAN system on both stem lodging of winter wheat and incidence of powdery mildew as well as *Septoria tritici* blotch was confirmed.

Key words: ammonium, bioethanol, biofuel, depot, harvest index, nitrification.

INTRODUCTION

Insufficient nitrogen supply during vegetation period of cereals causes substantial reductions in grain yield (e.g. Dekic et al., 2014). High supply of nitrogen, however, can reduce grain yield of cereals due to increased risk of lodging (O´Donovan et al., 2011) resulting in decreased grain yield and deterioration of grain quality parameters (Wang et al., 2015).

One of feasible possibilities to reduce potential negative impacts of high nitrogen supplies on both environment and crop, should represent an injection of liquid nitrogen fertilizers into soil by the CULTAN (Controlled Uptake Long Term Ammonium Nutrition) system. The CULTAN system was recommended particularly based on its claimed higher efficiency of nitrogen use (Sommer and Scherer, 2009). Thus, with the CULTAN treatment, all nitrogen could be applied just in one dose using common liquid fertilizers (Sommer and Scherer, 2009; Peklova et al., 2012; Kubesova et al., 2014).

A use of the CULTAN system should also lead to significantly shortened stalk of cereals (Sommer and Scherer, 2009) which is according to Berry et al. (2004) important to reduce risk of stem lodging. Furthermore, differences in nitrogen uptake by CULTAN-treated plants should contribute to higher resistance of the plants to fungal diseases infestation. Thus, higher grain yield of CULTAN treated cereals compared to the ones treated with conventional nitrogen split application is expected (Sommer and Scherer, 2009).

A lower protein content in grain of spring barley and winter wheat treated with

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the CULTAN system compared to using of split nitrogen application (Sedlar et al., 2011; Kozlovsky et al., 2009, 2010) could be useful for bioethanol production (Sedlacek, 2011), i.e. by far the most widely used biofuel for transportation worldwide (Balat, 2011) and the most perspective present and future biofuel (Hromadko et al., 2010).

According to Sommer and Scherer (2009) the nitrogen fertilizer should not be applied using the CULTAN system to cereals until the end of tillering (BBCH 29). However, according to the findings of Kozlovsky et al. (2010) the nitrogen injection just at the end of tillering (BBCH 29) leads, under conditions of the Czech Republic, to the deterioration of baking quality of grain of CULTAN-treated winter wheat compared to the winter wheat treated with nitrogen split into three doses. Therefore, according to the findings of Kozlovsky et al. (2010) the nitrogen fertilization by the CULTAN system should be carried out at an earlier date in spring.

The aim of this study was to compare winter wheat development using the injection of nitrogen fertilizer (CULTAN system) with the nitrogen split application on soil surface under field conditions of the Czech Republic. Higher grain yield as well as decrease in both stem lodging and fungal diseases incidence are assumed while using the CULTAN system compared to the nitrogen split application. Potential utilization of the CULTAN system to starch production and the resulting bioethanol production was also evaluated even though the cultivar Sulamit is of breadmaking quality.

MATERIAL AND METHODS

Small-plot field experiments with winter wheat (*Triticum aestivum* L.) cultivar Sulamit (sowing density of 450 seeds per m^2, size of a fertilized plot – 39 m^2, 15 m^2 harvested plot) were conducted during 2009-2013 at three sites with different soil-climatic conditions in the Czech Republic (Central Europe): Hněvčeves (50°18'46.269"N,15°42'51.552"E), Humpolec (49°32'49.604"N, 15°21'6.405"E) and Ivanovice na Hané (49°18'34.209"N, 17°5'18.753"E). At the Ivanovice na Hané site the experiment was conducted only during the years 2009-2012. Characteristics of the experimental sites are given in e.g. Kozlovsky et al. (2009).

Split applications of nitrogen 43+87+20 kg N/ha and 60+90+50 kg N/ha respectively in calcium ammonium nitrate (27% N) applied on soil surface were compared with the injection of urea ammonium nitrate solution (30% N) applied in one dose of fertilizer with the GFI 3A injection machine (Maschinen und Antriebstechnik, Germany) to a depth of 5-10 cm into the soil. Both treatments were evaluated in two amounts of nitrogen fertilizers: 150 kg N/ha and 200 kg N/ha. Experimental scheme is given in Table 1. The fertilizer types used in the experiment were specifically chosen to make possible putting our results effortlessly into practice.

Contrary to the findings of Sommer and Scherer (2009), the CULTAN fertilization in our experiments was carried out at the beginning of spring vegetation, according to preliminary recommendations of Kozlovsky et al. (2010) for the soil-climatic conditions of the Czech Republic.

Table 1. Fertilizer treatment, N amounts and timing of N-fertilizers application.
CAN – Calcium Ammonium Nitrate (27% N); UAN – Urea Ammonium Nitrate (30% N)

Term of N-fertilization /Treatment	Split N 150	CULTAN 150	Split N 200	CULTAN 200
Beginning of spring vegetation	43 kg (CAN)	150 kg (UAN)	60 kg (CAN)	200 kg (UAN)
Third node becomes visible (BBCH 33)	87 kg (CAN)		90 kg (CAN)	
Beginning of heading (BBCH 52)	20 kg (CAN)		50 kg (CAN)	
Total nitrogen dosage per ha	150 kg	150 kg	200 kg	200 kg

The number of plants per m^2 was determined at the beginning of erect growth (BBCH 30). The number of ears per m^2, number of grains per ear (from a sheaf of 50 ears), thousand grain weight and length of stalk were determined prior to harvest. The harvest was done by a small-plot combine harvester; grain yield was determined by weighing grains from individual plots and converting it into 14% moisture.

Total nitrogen concentration in grain was determined by the Kjeldahl method using the Vapodest 50s (Gerhardt, Germany). Nitrogen percentage was then multiplied by the 5.7 factor for calculating grain protein content. Although the cultivar Sulamit is of breadmaking quality, the grain starch content was determined using the NIR OmegAnalyzer G (Bruins Instrument, USA) to evaluate a potential utilization of the CULTAN system to bioethanol production. Starch yield (protein yield) was calculated as a product of grain yield (t/ha) and grain starch content (%) (grain protein content (%) respectively).

Incidence of stem lodging and plant diseases during vegetation period was evaluated using the 1-9 scale (9 – undetected, 1 – all-out occurrence). A visual method according to external symptoms was used. Incidence of two fungal diseases was assessed: *Septoria tritici* blotch and powdery mildew. The *Septoria tritici* blotch caused by *Mycosphaerella graminicola*, is one of the most destructive foliar diseases of wheat across the world (Mergoum et al., 2013), under conditions of the central Europe, especially when a forecrop of winter wheat is a winter rape (Kuzdralinski et al., 2015). The powdery mildew, caused by *Blumeria graminis* (syn. *Erysiphe graminis*), is one of the major diseases of wheat worldwide (Liu et al., 2001).

Samples of aboveground biomass at the growth stages of BBCH 30 (beginning of erect growth), BBCH 45 (boot stage) and BBCH 51 (heading 1/4 complete) were taken from a 0.25 m^2 area. Dry matter content in aboveground biomass was expressed as a ratio of dry matter weight to a weight of sample of actual moisture.

Statistical analysis of the data was carried out using the Statistica version 12 (StatSoft, USA). A standard analysis of variance (ANOVA) procedures with the Tukey test was used to calculate significant differences between the individual treatments of nitrogen fertilization at a $p<0.05$ level of significance.

RESULTS

As shown in Table 2, the number of plants per m^2 determined at the beginning of erect growth (BBCH 30) as well as the number of ears per m^2 did not significantly differ among the nitrogen treatments.

Number of grains per ear was significantly lower at the CULTAN treatment in comparison to the split application of nitrogen, only when 150 kg N/ha was applied (Table 2). No significant differences between the nitrogen split application and the CULTAN system in both thousand grain weight and grain yield were recorded (Tables 2 and 3).

Table 2. Yield formation. The results are presented across the three sites and all experimental years

Treatment	Split N 150	CULTAN 150	Split N 200	CULTAN 200
Plants per m^2	382	382	385	385
Ears per m^2	604	608	605	609
Grains per ear	44^{a}	42^{b}	43^{ab}	43^{ab}
Thousand grain weight (g)	44.4	44.5	44.3	44.6

Notes: Values within the row marked either with the same letter or without exponents are not statistically different at $p<0.05$.

CULTAN-treated winter wheat achieved significantly lower grain protein content compared to the winter wheat fertilized with the nitrogen split application. However, sufficient baking quality of grain regarding the grain protein content of the CULTAN-treated winter wheat was always achieved. As a result, significantly lower protein yields at the CULTAN treatment compared to the nitrogen split application were recorded (Table 3). Despite significantly higher starch content in grain of the CULTAN-treated winter wheat compared to the one fertilized with the split application of nitrogen, significant differences between the CULTAN system and the nitrogen split application in starch yield were not confirmed (Table 3).

Table 3. Qualitative parameters of grain, grain yield, protein yield and starch yield. The results are presented across the three sites and all experimental years

Treatment	Split N 150	CULTAN 150	Split N 200	CULTAN 200
Grain yield (t/ha)	8.85^{ab}	8.54^{b}	8.90^{a}	8.88^{a}
Grain protein content (%)	13.6^{a}	12.7^{b}	14.0^{c}	13.1^{d}
Protein yield (t/ha)	1.18^{ac}	1.08^{b}	1.24^{c}	1.15^{a}
Grain starch content (%)	65.9^{a}	66.8^{b}	65.4^{c}	66.4^{d}
Starch yield (t/ha)	5.85	5.73	5.84	5.93

Notes: Values within the row marked either with the same letter or without exponents are not statistically different at p<0.05.

As shown in table 4, length of stalk, harvest index and dry matter content in aboveground biomass at the BBCH 30, BBCH 45 and BBCH 51 growth stages were not significantly different between the CULTAN treatments and the nitrogen split application.

No significant differences between the CULTAN system and the split nitrogen application in stem lodging were found (Table 4), on the other hand, the stem lodging in our experiments occurred only in a single year at just one experimental site. No significant differences in this case were recorded either. Neither powdery mildew nor *Septoria tritici* blotch incidence significantly differed among the nitrogen treatments (Table 4).

Table 4. Length of stalk, harvest index and dry matter (DM) content in aboveground biomass of winter wheat. Incidence of stem lodging and fungal diseases is evaluated using the 1- 9 scale (9 – undetected, 1 – all-out occurrence). The results are presented across the three sites and all experimental years.

Treatment	Split N 150	CULTAN 150	Split N 200	CULTAN 200
Length of stalk (cm)	86.8	86.0	87.1	87.3
Harvest index	0.545	0.546	0.537	0.537
Stem lodging	8.5	8.5	8.5	8.5
DM content at BBCH 30 (%)	18.2^{ab}	18.4^{b}	17.9^{a}	18.0^{a}
DM content at BBCH 45 (%)	22.1	21.8	21.8	22.2
DM content at BBCH 51 (%)	22.0	23.0	22.8	23.2
Septoria tritici blotch incidence	6.9	6.9	6.9	6.9
Powdery mildew incidence	8.1	8.3	8.2	8.1

Notes: Values within the row marked either with the same letter or without exponents are not statistically different at p<0.05.

Increased dosage of nitrogen (200 kg N/ha) led to significant increase in grain protein content both at the CULTAN system and the nitrogen split application; grain yield as well as protein yield were increased just at the CULTAN system. Increased dosage of nitrogen did not bring any other benefits in our experiments.

DISCUSSION

Although number of grains per ear was significantly lower while using the CULTAN system compared to the nitrogen split application, this decrease was only 3.6%. In addition, according to Jockovic et al. (2014) number of grains per ear could not have a significant influence on grain yield.

Insignificant differences among nitrogen treatments in number of ears per m^2 could be explained by high sowing density (450 seeds per m^2).

Dimmock and Gooding (2002) stated that decreased soil nitrogen availability at latter growth stages of winter wheat was connected with shortening of grain filling resulting in decreased thousand grain weight. Racz et al. (2015) also state that the thousand grain weight is a heritable trait less influenced by environment. Due to insignificant differences in thousand grain weight among the nitrogen treatments in our experiments, considerable prolongation of soil nitrogen availability while using the CULTAN system cannot be assumed.

Although the CULTAN treatment led to decrease in grain protein content compared to the nitrogen split application, no significant differences between the CULTAN treatment and the nitrogen split application in grain yield were recorded. This phenomenon is in agreement with the findings of Janusauskaite (2013) who stated that nitrogen rate splitting significantly increased grain protein content in comparison with a single application before sowing of spring triticale. However, the grain yield was not significantly influenced by the nitrogen rate splitting (Janusaiskaite, 2013). On the other hand, the decrease in grain protein content in our experiments led to significantly lower protein yield at the CULTAN treatments compared to the nitrogen split applications.

Significantly higher grain starch content with the CULTAN treatment compared to the nitrogen split application could be explained by significantly lower protein content in grain of the CULTAN-treated winter wheat compared to the one fertilized with the nitrogen split application. These results are in accordance with the findings of Asthir et al. (2014). Despite significantly higher grain starch content achieved while using the CULTAN system compared to the nitrogen split application, no significant differences in starch yield between the nitrogen treatments were recorded, which could be explained by insignificant differences in grain yield between the nitrogen treatments. This finding corresponds with the results of Sedlacek (2011) who stated that starch yield is affected by grain yield rather than by the grain starch content. Stem lodging is influenced particularly by length of stalk (Wiersma et al., 2011), harvest index (Foulkes et al., 2011), number of ears per m^2 as well as nitrogen uptake before anthesis (Webster et Jackson, 1993).

The length of stalk was not significantly influenced by the nitrogen treatment. Thus, the findings of Sommer and Scherer (2009) who explain shorter stalk of cereals as a result of the CULTAN fertilization were not confirmed. On the other hand, Sommer and Scherer (2009) assume an uptake of nitrogen by CULTAN-treated plants particularly in ammonium form inhibiting elongation growth (Skobeleva et al., 2011); however, according to the results of Flisch et al. (2013) and Sedlar et al. (2013) significantly higher ammonium uptake by the CULTAN-treated plants compared to the ones treated with the nitrogen split application cannot be assumed.

The insignificant differences in harvest index among the nitrogen treatments could be explained by optimal nitrogen doses applied in our experiments, which is in accordance with the findings of Amanullah (2014).

Due to ammonium uptake by the CULTAN-treated plants, Sommer and Scherer (2009) stated less osmotic pressure of plant cells due to less nitrate concentration in cells of the CULTAN-treated plants compared to plants treated with the split nitrogen fertilization. As a result, the cell wall became thicker at the CULTAN treatment compared to the nitrogen split application, resulting in higher resistance of CULTAN-treated plants to fungal diseases infestation (Sommer and Scherer, 2009). Less turgor of cells of the CULTAN-treated plants was also recorded by Pavlik et al. (2010) in a pot experiment with maize. In our experiments though, no significant differences between the CULTAN system and the nitrogen split application in dry matter contents were found, which could explain insignificant differences in both powdery mildew and *Septoria tritici* blotch incidence between the CULTAN-treatment and the nitrogen split application. This phenomenon could be explained by the insufficient inhibition of nitrification of injection-applied nitrogen fertilizer, which is in accordance with the results of Flisch et al. (2013) and Sedlar et al. (2013).

Furthermore, according to the results of Sedlar et al. (2015), nitrogen content in aboveground biomass of winter wheat at the BBCH 45 (boot stage) as well as BBCH 51 (heading 1/4 complete) growth stages was not significantly different between the CULTAN system and the nitrogen split application. Since none of the above mentioned parameters relating to stem elongation differed between the CULTAN system and the nitrogen split application, significantly less stem lodging as a result of the CULTAN system cannot be assumed.

Increased dosage of nitrogen (200 kg N/ha) led to significant increase in grain protein content both at the CULTAN system and the nitrogen split application. Grain yield as well as protein yield were increased only at the CULTAN system. These results are in agreement with the findings of Neugschwandtner et al. (2014) who stated that nitrogen concentrations and nitrogen yields throughout crop growth are increased by increasing rates of nitrogen fertilization.

CONCLUSIONS

Number of ears per m^2 and thousand grain weight were not significantly different between the CULTAN system and the nitrogen split application. Number of grains per ear was significantly lower at the CULTAN treatment in comparison with the nitrogen split application when 150 kg N/ha was applied. This decrease, however, led to insignificant difference in grain yield between the CULTAN system and the nitrogen split application. Despite higher starch content in grain of the CULTAN-treated winter wheat compared to the one treated with the nitrogen split application, no significant differences among nitrogen treatments in starch yield were recorded. Potentially advantageous utilization of the CULTAN system to starch production and the resulting bioethanol production cannot be assumed.

Except for decrease in grain protein content due to the CULTAN system, no other parameters can indicate prolonged soil nitrogen availability as a result of the nitrogen injection compared to the conventional nitrogen split application.

Incidence of stem lodging, *Septoria tritici* blotch, as well as powdery mildew, was not significantly influenced by the nitrogen treatment, which can be explained by the same level of nitrate uptake by both the CULTAN-treated winter wheat and the one treated with the nitrogen split application.

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