Uptake and Utilization of Different Nitrogen Forms by Wheat Plants

Mgr. Ivana Raimanová
(Summary Ph.D. Thesis)

Supervisor: Ing. Jan Haberle, CSc., VÚRV, v.v.i., Praha
Co-supervisor: Ing. Marie Trčková, VÚRV, v.v.i., Praha

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1. Introduction

In spite of the Green Revolution's success, the growing world population together with the degradation of agricultural land and its decrease create pressure on increasing the food production rate and the volume of crop production. Economic reasons and the whole society emphasis on environmental protection lead to the search of new possibilities to increase the efficiency of necessary inputs, esp. energy, nitrogen fertilizers and plant protection products.

Economically significant wheat species (*Triticum aestivum* L. and *Triticum durum* Desf.) belongs with its area to the most grown crops in the world and they ensure an important part of the food of the world's population. Agro-ecological conditions under which wheat is grown, ranges from semiarid areas to areas with frequent precipitation, from tropics to cool areas in high latitudes with short growing season. Crop systems include various methods of plant nutrition and irrigation, different crop rotations and various soil treatment.

This range of soil-climatic and production conditions also corresponds to a broad range of soil nitrogen availability and general conditions for nutrient uptake and utilization. Amount and availability of individual forms of nitrogen for plants varies during their ontogenetic development as a consequence of dynamics of soil nitrogen transformation and depending on specific weather conditions.

Nitrogen is an essential macronutrient to obtain the required yield and quality of grown plants acting in interaction with other nutrients and water availability. To increase efficiency of utilization of nitrogen and other nutrients it is necessary to learn and interconnect the knowledge of physiological, biochemical and molecular mechanisms controlling nitrogen uptake, assimilation and utilization.
2. **Objectives**

The subject of this thesis was determined by the need to utilize new findings concerning nitrogen uptake and utilization by plants in applied agricultural research. Its main aim was to determine the influence of ammonium, nitrate and amide forms of nitrogen nutrition on nitrogen uptake by roots and leaves of common wheat and its subsequent utilization.

Solving of selected problems was divided into the following partial aims:

1. To determine the influence of nitrate and ammonium nitrogen forms and their combination on the growth of wheat plants and to characterize differences in nitrogen metabolism including effect of low pH on studied characteristics.

2. To determine, using kinetic parameters for uptake, intra- and inter-species differences in the efficiency to receive and utilize nitrate and ammonium forms of nitrogen.

3. To determine the rate of uptake and subsequent translocation of individual forms of nitrogen and to determine the temperature dependence of the uptake.

4. To obtain new findings about uptake and utilization of various forms of nitrogen during the foliar application and about the subsequent translocation of nitrogen in the plant.

5. To determine the utilization efficiency of leaf-applied urea in late development phase for the wheat grain production and to determine the influence of the common application with plant protection products on urea uptake and utilization.
3. Results and Discussion

1. Effect of nitrate and ammonium and their combination on the growth of wheat plants including effect of low pH

The mineral nitrogen content in soil and its availability for plants changes quickly during vegetation depending on physical-chemical properties of soil (pH, O\textsubscript{2} content, water availability) and activity of soil microorganisms. Simultaneously proportion of individual forms of nitrogen (NH\textsubscript{4}\textsuperscript{+}: NO\textsubscript{3}\textsuperscript{-} ratio) changes. Plants can utilize both forms as nitrogen source but in spite of that the preference of one form only was observed in many plant species. At cereals preferably nitrates are generally utilized, with the exception of rice.

At sensitive plant species, "toxicity symptoms" of ammonium ion are often observed. The main toxicity symptoms are reduced growth rate, especially of roots, decrease of R/S ratio or lower content of essential cations like potassium, calcium or magnesium (Marschner, 1995; Britto and Kronzucker, 2002).

Wheat, like some other species of cultivated plants, belongs to the species sensitive to toxic effect of ammonium (Cramer and Lewis, 1993; Britto et al., 2001), which was also confirmed in our experiments. Wheat plants responded to the presence of ammonium ion as the only nitrogen source significantly negatively. The production of root biomass was decreased and their development was limited which was manifested by the significant decrease of R/S ratio when compared to plants grown with nitrate or mixed nutrition.

One of possible causes of these symptoms is the lack of carbon. Ammonium, in contrast to nitrate, is largely assimilated in roots. Assimilation of ammonium ions and development of root system are thus competing for carbon skeletons and energy and therefore plants grown only with ammonium nutrition have a significantly lower R/S ratio.

Decreased content of essential cations (K\textsuperscript{+}, Ca\textsuperscript{2+}; Mg\textsuperscript{2+}) is another observed symptom of the effect of NH\textsubscript{4}\textsuperscript{+}. In our experiments, the uptake of ammonium ion as the only nitrogen source significantly negatively influenced the content of these cations, both in shoots and roots of wheat plants (Table 1). At the same time, their lower content was also determined
in plants grown with mixed nutrition. The uptake and subsequent translocation of essential cations are thus significantly influenced by the form of taken up nitrogen. We observed that together with lower content of essential cations the plants from the ammonium treatment have higher content of total nitrogen when compared to the nitrate and mixed treatments. Similar findings were also reported at other plants (Cramer and Lewis, 1993; Ruan et al., 2000).

**Tab. 1** Essential cations content in wheat plants (*Triticum aestivum*) grown for 3 weeks in nutrient solution with different N form. Average ± SD; P=0.05; the red colour - statistic valuation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrate</th>
<th>Mixed</th>
<th>Ammonium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K</strong> (mg K.g⁻¹ DW)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots (P=0.0001; F=103.77; Df=11)</td>
<td>52.16±4.82 <strong>c</strong></td>
<td>40.09±2.36 <strong>b</strong></td>
<td>15.39±1.29 <strong>a</strong></td>
</tr>
<tr>
<td>Shoots (P=0.0001; F=116.52; Df=14)</td>
<td>53.09±3.05 <strong>c</strong></td>
<td>47.52±0.92 <strong>b</strong></td>
<td>32.28±0.88 <strong>a</strong></td>
</tr>
<tr>
<td><strong>Ca</strong> (mg Ca.g⁻¹ DW)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots (P=0.0001; F=21.17; Df=14)</td>
<td>2.05±0.29 <strong>b</strong></td>
<td>1.38±0.22 <strong>a</strong></td>
<td>0.95±0.22 <strong>a</strong></td>
</tr>
<tr>
<td>Shoots (P=0.0001; F=162.42; Df=16)</td>
<td>4.15±0.28 <strong>c</strong></td>
<td>3.19±0.08 <strong>b</strong></td>
<td>1.66±0.23 <strong>a</strong></td>
</tr>
<tr>
<td><strong>Mg</strong> (mg Mg.g⁻¹ DW)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots (P=0.0001; F=35.73; Df=12)</td>
<td>2.07±0.33 <strong>b</strong></td>
<td>1.08±0.19 <strong>a</strong></td>
<td>0.66±0.11 <strong>a</strong></td>
</tr>
<tr>
<td>Shoots (P=0.0001; F=35.01; Df=14)</td>
<td>2.21±0.35 <strong>c</strong></td>
<td>1.62±0.12 <strong>b</strong></td>
<td>1.04±0.10 <strong>a</strong></td>
</tr>
</tbody>
</table>

Another toxicity symptom observed in many plants is the uptake of excessive amounts of ammonium ions and their accumulation in plant tissues. As it has recently been found, the main difference between the species sensitive and tolerant to the effect of ammonium is the ability to regulate the uptake of ammonium through the plasmatic membrane. In sensitive species this regulation is disturbed, leading to the accumulation of excessive amounts of ammonium in the cytosol. Ammonium ions are subsequently secreted outwards against high concentration gradient, and consequently high amounts of energy are consumed (Britto et al., 2001). On the other hand, uptake of ammonium in tolerant species is more slowly. Their concentration in cytosol increases depending on ambient
concentration in the rhizosphere (Wang et al., 1993; Kronzucker et al., 1995) and reverse release of ammonium ions occurs there only passively based on the concentration gradient (Britto et al., 2001).

When compared to the nitrate fed plants in ammonium fed plants higher content of ammonium ions in roots and also in the oldest leaf were found (Fig. 1). This increased content of ammonium ions in the oldest parts of the plant lead us to the hypothesis that another symptom of toxicity of ammonium ions could be a more rapid induction of senescence of oldest leaves. Accumulation of ammonium ions in tissues of senescing wheat leaves has already been described (Thomas, 1978; Peeters and van Laere, 1992). Senescence of leaves is accompanied by degradation of chlorophyll. Older leaves contain less chlorophyll than younger ones. Also in our experiments, the content of chlorophyll changed depending on the leaf age. In plants grown with ammonium nutrition a lower content of chlorophyll was observed in the oldest leaf and also in fully developed leaves, but found differences were not statistically significant.

![Graph showing content of ammonium ions in different plant parts of wheat grown for 3 weeks at ammonium (NH$_4^+$ pH-stat – pH stabilized at 5.8 – 6.0) or nitrate supply (NO$_3^-$ pH-stat – pH stabilized at 4.4 – 4.6). Average ± SD.

Fig. 1 Content of ammonium ions in different plant parts of wheat grown for 3 weeks at ammonium (NH$_4^+$ pH-stat – pH stabilized at 5.8 – 6.0) or nitrate supply (NO$_3^-$ pH-stat – pH stabilized at 4.4 – 4.6). Average ± SD.

The uptake of ammonium and their assimilation is associated with the release of protons, which causes strong acidification of rhizosphere
(Marschner, 1995; Smart and Bloom, 1998). Low pH is considered to be one of main reasons of symptoms of ammonium toxicity. It was observed in many plant species that toxicity symptoms were significantly lower when higher pH has been maintained in nutrient medium (Bloom et al., 1993; Schortemeyer et al., 1993). It is therefore possible that plant species where alleviation of symptoms occurred at higher pH suffer not only from toxicity of ammonium ions, but also from the stress induced by the acidity of nutrient medium.

These observations fully corresponded with our results. Wheat plants are a very sensitive species to the effect of low pH. In plants grown with ammonium nutrition the production of root biomass was stimulated by higher pH and at the same time the R/S ratio increased almost twice. Similar results were reported by Schortemeyer et al. (1993) in maze plants, where at pH 4 the production of dry matter of roots in ammonium nutrition was hardly half when compared to the nitrate production, but at pH 7 the production of root biomass was higher in the ammonium nutrition than in the nitrate one. On the other hand, in plant species adapted to the growth in acid soils and preferring ammonium as a nitrogen source, the better root growth was observed in the ammonium nutrition than in the nitrate one, even at low pH (Garnett and Smethurst, 1999).

In addition to the stimulation of root growth, higher pH of the nutrient solution had also a positive effect on the uptake and resulting content of essential cations (Fig. 2). In plants with ammonium as the only source of nitrogen the increase of pH lead to the increase of the content of all three studied cations (K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\)) in tissues of both leaves and roots. In nitrate fed plants the differences in ion contents were less distinctive, nevertheless the plants cultivated at lower pH contained significantly less of these cations. Therefore, it is obvious that uptake of cations is inhibited not only during the uptake of ammonium ions, but at the same time it is significantly regulated depending on pH of the nutrient solution. The pH value of the rhizosphere influences the activity of channels for the uptake of ions, esp. in the case of potassium.
Fig. 2  Potassium content in root and shoot of wheat grown for 3 weeks under ammonium supply; pH-stat – stabilized pH at 5.8 – 6.0. Average ± SD; (roots: P=0.0003; F=55.99; Df=7; shoots: P=0.0006; F=42.54; Df=7).

The toxic effect of ammonium ions in most of the plants was partly or fully suppressed as long as the plants were grown in the medium where both forms of nitrogen were available (Wang and Below, 1995; Schortemeyer et al., 1997). In our experiments, the wheat plant from the mixed treatment had significantly higher production of shoot biomass and their root growth was comparable to the plants from the nitrate treatment. In spite of the observed negative effects of ammonium on the grown plants, the use of the ammonium nitrogen fertilizers represents one of the possibilities leading to the reduction of cost and to the reduction of the pollution of both underground and surface waters by nitrates. The popular system CULTAN (Controlled Uptake Long-Term Ammonium Nutrition), developed in 90th in Germany (Sommer, 2000) is an attempt for the practical solution of this problem.

2  Intra- and inter-species differences in the rate of uptake between the nitrate and ammonium forms of nitrogen

Wheat, rice and maze belong to the most important crops in the world that ensure a substantial part of the food production. Most of wheat varieties currently grown in this country were selected by breeders with
regard to the yield level, technological quality of grain (i.e. content and structure of storage proteins), resistance to fungal diseases and to a less extent also to frost resistance and resistance to drought. Regarding the rising prices of fertilizers and the necessity to ensure food production for the constantly growing human population it will be necessary to select genotypes where the required yield will be provided even under low N fertilization input (Le Gouis et al., 2000; Delmer, 2005) and consequently genotypes with greater efficiency of nitrogen utilization (Hirel et al., 2007).

The uptake of both forms of nitrogen from soil is mediated by 2 types of transport systems depending on their concentration. High-affinity transport systems (HATS) ensure active uptake at low outer ion concentrations (< 0.5 mM) and show higher affinity to the substrate. At high outer concentration (> 0.5 mM) low-affinity transport systems (LATS) are triggered. The rate of uptake linearly increases with ambient concentration. In field conditions concentrations of both ions during vegetation significantly change, but conditions for functioning of LATS mostly only occur during a limited period (e.g. after fertilizer applications). Therefore, the main importance for the uptake of both N forms have rather HATS.

Use of uptake parameters $V_{\text{max}}$ and $K_m$ characterizing high-affinity uptake system appears to be an appropriate marker of the ability to uptake N from the environment. Kinetic parameters were studied in many plant species (Siddiqi et al., 1990; Lainé et al., 1993; Mäck and Tischner, 1994; Crawford and Glass, 1998; Malagoli et al., 2004) nevertheless their comparison generally is not possible because of different experimental conditions. In this work kinetic parameters for the uptake of nitrates and ammonium ions at twelve varieties of wheat ($Triticum aestivum$ and $Triticum durum$) were compared. In both studied species significantly higher $V_{\text{max}}$ value was found for ammonium uptake (16.9 and 16.4 μmol of $NH_4^+$·g$^{-1}$ FW·h$^{-1}$) than for nitrate uptake (6.5 and 5.1 μmol of NO$_3^-$·g$^{-1}$ FW·h$^{-1}$), and simultaneously higher affinity (and thus lower $K_m$) for nitrate. It shows better acquiring ability for nitrate uptake in both species.

In the intra-species comparison, higher differences in the nitrate uptake were found. In common wheat the Portuguese cultivar Roxo differed from the Canadian cultivar AC Reed belonging to the same botanical group. In hard wheats the Mexican cultivar Mojo 2 was significantly different from
the other six predominantly southern European cultivars. Differences were observed for the value $K_m$ as well. At the same time, it was found that values of both $V_{\text{max}}$ and $K_m$ are significantly greater when compared to the hard wheat varieties (Tab. 2).

Tab. 2 Inter- and intra-species differences in $V_{\text{max}}$ and $K_m$ characterizing high-affinity transport system for nitrate. Average ± SD; P=0.05; the red colour – statistical valuation; bold type = inter-species differences.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>$V_{\text{max}}$ ($\mu\text{mol NO}_3\cdot\text{g}^{-1}\cdot\text{FW.h}^{-1}$)</th>
<th>$K_m$ ($\mu\text{M}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Triticum aestivum</em></td>
<td>6.54±1.33 b</td>
<td>26.85±3.32 b</td>
</tr>
<tr>
<td>Roxo</td>
<td>5.13±0.72 a</td>
<td>22.4±6.9</td>
</tr>
<tr>
<td>AC Read</td>
<td>7.78±1.44 b</td>
<td>30.1±11.5</td>
</tr>
<tr>
<td>Pacifik</td>
<td>5.53±0.74 ab</td>
<td>25.9±7.4</td>
</tr>
<tr>
<td>Munk</td>
<td>6.55±0.46 ab</td>
<td>24.6±8.9</td>
</tr>
<tr>
<td>Sandra</td>
<td>7.36±0.96 ab</td>
<td>31.2±12.7</td>
</tr>
<tr>
<td><em>Triticum durum</em></td>
<td>5.11±1.59 a</td>
<td>20.67±2.35 a</td>
</tr>
<tr>
<td>Zenit</td>
<td>4.33±0.28 a</td>
<td>20.8±11.4</td>
</tr>
<tr>
<td>Marmilla</td>
<td>5.14±0.75 a</td>
<td>24.2±3.0</td>
</tr>
<tr>
<td>Saadi</td>
<td>5.12±1.05 a</td>
<td>16.7±4.9</td>
</tr>
<tr>
<td>Kharkovskaya 21</td>
<td>3.59±0.86 a</td>
<td>21.7±2.1</td>
</tr>
<tr>
<td>Lyudmila</td>
<td>4.66±1.41 a</td>
<td>-</td>
</tr>
<tr>
<td>Valbelice</td>
<td>4.35±0.80 a</td>
<td>21.7±3.5</td>
</tr>
<tr>
<td>Mojo 2</td>
<td>7.90±0.79 b</td>
<td>19.1±5.5</td>
</tr>
</tbody>
</table>

*- Statistical analysis of *T. aestivum* (P=0.0175; F=4.21; Df=19). Statistical analysis of *T. durum* (P=0.0010; F=6.39; Df=24). Statistical analysis of *T. aestivum* x *T. durum* (P=0.0030; F=9.87; Df=44 pro $V_{\text{max}}$ a P=0.0099; F=10.59; Df=10 pro $K_m$).

Much smaller statistically insignificant differences between species and varieties were observed in the uptake of ammonium ions which was influenced by the generally worse development of the root system grown with ammonium nutrition and thus greater variability within one variety (Tab.3).

Found intra- and inter-species differences in the rate of uptake are further modified by the development of the root system. Generally it can be
said that at the same rate of uptake there is a greater uptake capacity (= µmol N.g⁻¹.plant⁻¹.h⁻¹) in varieties with higher R/S ratio. In all studied varieties of both wheat species significantly higher R/S ratio was found in plants from the nitrate treatment when compared to the plants from the ammonium treatment.

**Tab. 3** Inter- and intra-species differences in \( V_{\text{max}} \) a \( K_m \) characterizing high-affinity transport system for ammonium ions. Average ± SD; P=0,05; the red colour – statistical valuation; bold type = inter-species differences.

<table>
<thead>
<tr>
<th>Odrůda</th>
<th>( V_{\text{max}} ) (µmol NH₄⁺.g⁻¹ FW.h⁻¹)</th>
<th>( K_m ) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triticum aestivum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roxo</td>
<td>16,57±1,00</td>
<td>53,2±6,5</td>
</tr>
<tr>
<td>AC Read</td>
<td>14,89±2,62</td>
<td>35,7±9,7</td>
</tr>
<tr>
<td>Pacifik</td>
<td>18,18±1,09</td>
<td>55,6±17,5</td>
</tr>
<tr>
<td>Munk</td>
<td>16,43±2,44</td>
<td>34,3±3,6</td>
</tr>
<tr>
<td>Sandra</td>
<td>19,56±3,04</td>
<td>39,4±0,9</td>
</tr>
<tr>
<td><strong>Triticum durum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zenit</td>
<td>17,08±4,74</td>
<td>50,1±14,9</td>
</tr>
<tr>
<td>Marmilla</td>
<td>18,35±5,09</td>
<td>44,1±2,8</td>
</tr>
<tr>
<td>Saadi</td>
<td>16,63±3,59</td>
<td>32,8±2,3</td>
</tr>
<tr>
<td>Kharkovskaya 21</td>
<td>16,69±2,63</td>
<td>38,5±7,1</td>
</tr>
<tr>
<td>Lyudmila</td>
<td>13,45±2,65</td>
<td>38,7±6,5</td>
</tr>
<tr>
<td>Valbelice</td>
<td>13,02±2,48</td>
<td>34,8±12,5</td>
</tr>
<tr>
<td>Mojo 2</td>
<td>20,11±3,61</td>
<td>33,0±6,8</td>
</tr>
</tbody>
</table>

*- Statistical analysis of *T. aestivum* (P=0,1775; F=7,84; Df=18). Statistical analysis of *T. durum* (P=0,2045; F=1,56; Df=28). Statistical analysis of *T. aestivum* x *T. durum* (P=0,6351; F=0,22; Df=47 pro \( V_{\text{max}} \) a P=0,3352; F=1,02; Df=11 pro \( K_m \)).

Obtained results indicate that the determination of uptake ability can be used as a useful tool for the selection and assessment of breeding material. It is valid provided that the comparison refers to the plants grown under exactly defined conditions. During breeding of varieties for the growth at low nitrogen supply, the cultivars with lower \( K_m \), higher \( V_{\text{max}} \) values and higher R/S ratio would be an appropriate genetic source.
3 The rate of uptake and translocation of individual forms of nitrogen

The rate of uptake of both nitrogen forms is not constant and differs not only between individual species or cultivars, but it is also significantly influenced by environmental factors (e.g. temperature, photosynthetically active radiation, diurnal rhythm or availability of the specific form of N) (Malagoli et al., 2004). At the same time, the uptake is regulated also at the plant level considering its current state, growth rate, ontogenetic development stage and especially N-status. The uptake of nitrate and ammonium ions thus undergoes a negative feed-back regulation given by the availability of the products of their assimilation in the plant (Cerezo et al., 2001; Glass et al., 2002; Loqué and von Wirén, 2004).

When comparing the rate of nitrate and ammonium uptake in plants grown under the same conditions in the nitrate or ammonium nutrition it was found that during the first hour the rate of nitrate uptake was slower approx. by ¼ than the rate of ammonium uptake as long as both ions were the only one available form of nitrogen (77.8 and 100.2 µmol.g⁻¹DW.h⁻¹). During the second hour the rate of uptake, esp. of ammonium ions, decreased substantially. The rate decrease could have been caused by the lower rate of assimilation of ammonium ions leading to their accumulation in roots and subsequent regulation of their uptake. This assumption corresponded also to the higher amount of absorbed nitrogen found in roots (Table 4). Nitrate before the transport to the above-ground part do not need to be assimilated into organic compounds. In the case of uptake of NO₃⁻ - form of N, the main transported form of N is nitrate itselfselves. This also corresponded to a significantly higher rate of translocation of N which, in our experiment, was absorbed in the form of nitrates (Table 4).

At the same time, substantial differences between N forms were observed in the sites where the acquired nitrogen was translocated. The highest portion of nitrogen acquired in the form of nitrate was translocated to mature leaves, where assimilation occurs. This also generally corresponded to the highest activity of nitrate reductase in these leaves. Smaller part was found in the prematuring growing leaves. In case of
ammonium ions, the acquired nitrogen was assimilated already in roots and subsequently preferably transported to the developed leaves (Table 4).

**Tab. 4** Dependence of N allocation in 3-week wheat plant on N form supply Average ± SD; P=0.05; red colour – statistical valuation.

<table>
<thead>
<tr>
<th>N form</th>
<th>Plant part</th>
<th>Nitrate (% acquired 15N)</th>
<th>Ammonium (% acquired 15N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>Roots (P=0.0004; F=48.26; Df=7)</td>
<td>63.7±2.5 a</td>
<td>83.2±4.2 b</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Senescing leaves (P=0.0012; F=32.93; Df=7)</td>
<td>3.2±0.8 b</td>
<td>0.5±0.2 a</td>
</tr>
<tr>
<td></td>
<td>Full expanding leaves (P=0.0001; F=325.25; Df=7)</td>
<td>18.0±1.5 b</td>
<td>1.5±0.7 a</td>
</tr>
<tr>
<td></td>
<td>Developing leaves (P=0.0071; F=16.03; Df=7)</td>
<td>7.0±1.0 b</td>
<td>3.3±1.3 a</td>
</tr>
<tr>
<td></td>
<td>Sheaths (P=0.2164; F=1.91; Df=7)</td>
<td>8.1±0.8</td>
<td>9.3±1.2</td>
</tr>
</tbody>
</table>

It was observed in many previous studies that if both forms of N are available in the medium, the rate of nitrate uptake is negatively influenced (Siddiqi *et al.*, 1990; Macduff and Jackson, 1991; Camanes *et al.*, 2009). This finding was fully confirmed by our results. If the plants received both ions in the same concentration, nitrate uptake was significantly inhibited (only 17.7 µmol.g⁻¹DW.h⁻¹). The rate of ammonium uptake was not influenced at all by the presence of nitrate. The total nitrogen uptake from the mixed nutrient treatment was therefore approximately by 20 % higher when compared to both variants where nitrogen was present only in one form. Inhibition of nitrate uptake in the presence of ammonium ions is explained by the negative feed-back regulation by the products of assimilation of absorbed ammonium ions (Forde and Clarkson, 1999).

4 Temperature effects on uptake of individual nitrogen forms

All the factors influencing nutrient utilization by the plant, e.g. their availability in the soil, morphological characteristics of root system, and physiological and biochemical properties responsible for the uptake itself and assimilation, are influenced by temperature. In our latitudes, the
average daily temperature determines the start of spring vegetation and its termination in autumn, and therefore determines the length of vegetation period. Simultaneously, low soil temperature slows down the processes of mineralization of organically bound nutrients.

It is obvious from our results that uptake rates of all forms of N are linearly dependent on temperature (Fig. 3). In ammonium ions the equation \(y = 8.68x + 15.26; R^2 = 99.3\%\) is valid in the whole temperature range, the uptake of nitrate \(y = 2.31x + 7.28; R^2 = 97.6\%\) is valid only within the range 2 °C to 15 °C. There is a linear relationship even for a very slow uptake of urea \(y = 0.40x + 4.32; R^2 = 94.9\%\).

From the comparison of the rate of uptake of all three forms it follows that the ammonium uptake is significantly faster than nitrate uptake (~ 2.5x), and esp. when compared to urea (~ 10x) at lower temperatures. At 20 °C the differences in the uptake rate were already smaller, nitrate uptake was 1.8x and urea uptake 5x slower.

![Graph showing uptake rates of N forms](image)

**Fig. 3**  Effect of root zone temperature on rate of different N form uptake by wheat plant after 4 week cultivation. Average ± SD.

Similar results were obtained by Malagoli et al. (2004). They found that the rate of nitrate uptake in oilseed rape significantly increased up to 16 °C, then the increase was less. It was also proved by these authors
that temperature influences the activity of high-affinity transport systems much more than the low-affinity transport systems.

In addition to the uptake of nutrients, generally the low temperature of plants decreases the growth rate and often higher R/S ratio is observed (Garnett and Smethurst, 1999). According to some authors, limited uptake of N at low temperatures resulting in the decrease of N content in shoots may indicate higher efficiency of its utilization for biomass formation (Macduff and Jackson, 1991).

5 Uptake and utilization of various nitrogen forms in foliar application

The rate of input of individual forms of N, i.e. their penetration through the cuticle and subsequent uptake into epidermis cells and mesophyll differs according their chemical nature. Nitrates are acquired as anions, ammonium ions as cations and urea is probably acquired as a whole molecule, i.e. as a non-polar compound.

In non-polar compounds, the penetration through the cuticle is driven by a gradient of concentrations. The diffusion rate thus depends both on the substance concentration on the leaf surface and on the concentration inside the leaf (in apoplast) which is influenced by the mobility of the substance and by the rate of its uptake into epidermal and mesophyll cells (Ewert et al., 2000). In polar compounds also membrane potential given by the charge is added to the chemical potential. Cations generally pass through the cuticle more easily than anions (Tyree et al., 1990). In our experiment, it was proved that ammonium ions are taken up by leaves significantly easier than nitrate. The amount of nitrogen coming from the ammonium form was significantly higher than from nitrate both after 4 hours and 7 days after the application (28 and 88 % when compared to 23 a 52 %). It was the confirmation of the hypothesis that cations pass through the cuticle more readily and therefore are taken up in leaf cell more rapidly. Urea as a small non-polar molecule passes through the cuticle probably more rapidly than polar substances. However, the amount acquired by the wheat leaf was significantly lower when compared to ammonium ions and only a little higher when compared to nitrate. The rate of uptake is therefore probably limited at the level of transport through plasmatic membrane.
Great differences were also observed in the translocation of nitrogen acquired from its various forms. Higher amounts of taken up ammonium ions are toxic for the cell and therefore ammonium is quickly assimilated into organic compounds (Marschner, 1995; Tobin and Yamaya, 2001). The organic compounds are then transported into new developed leaves or growing ear. It was confirmed also in our experiment, where the greatest part of the nitrogen acquired from the ammonium form was found in growing parts of the plant. Assimilation of ammonium ions in leaves is not probably limited by the availability of carbon skeletons, in contrast to their assimilation in roots, and therefore the part of translocated N was substantially higher than for nitrate, when compared to the situation of ammonium uptake and assimilation in roots. The acquired nitrate can be in a large extent stored in vacuoles and assimilation is controlled by the demand of the plant. In our experiments, the great part of nitrogen (56.5 %) remained in the exposed leaf and the transport was performed especially into growing parts of the main stalk and tillers (Fig. 4). The proportion of translocated nitrogen from the amide form after 7 days was $\frac{2}{3}$ from the acquired amount.

![Fig. 4 Allocation of N in wheat plant 7 days after feeding. Average ± SD.](image)
The efficiency of utilization of leaf-applied urea in late development phase for the production of wheat grain and the influence of the simultaneous application with plant protection products on urea uptake and utilization

During the vegetative phase of the growth nitrogen is taken up by roots and after assimilation it is used for forming of cell structures in young growing organs (leaves, stalks and roots). When the plant has transited to the reproductive phase of the growth (after anthesis), the nitrogen is remobilized and translocated from these organs into a forming grain. Part of nitrogen used for the formation of grain comes from its uptake after beginning of flowering. The proportion of remobilized and taken up N after anthesis differs between individual plant species and is influenced by many factors: availability of N in various stages of development, proper timing of fertilizer application and environment conditions (e.g. amount of photosynthetically active radiation or precipitation and soil humidity). In wheat 60 – 95 % of nitrogen used for the formation of grain comes from remobilization of nitrogen stored in roots and leaves (Barneix, 2007; Habash et al., 2006). Smaller part comes from the nitrogen uptake in the period of grain formation.

The uptake of nutrients by roots quickly rises in the period of intensive growth (during stem elongation), culminates at beginning of flowering and then decreases, more rapidly in plants well supplied with nitrogen (Trčková and Kamínek, 2000). The uptake of nitrogen from soil is then substantially limited. The ability of leaves to uptake and metabolize nutrients is preserved for a longer time and does not decrease until the process of aging. In wheat it was observed that leaves were metabolically active and therefore able to utilize the taken up nitrogen at least 10 – 15 days after anthesis (Raimanová and Trčková, 2007).

Under insufficient nitrogen nutrition a yield decrease occurs and as a rule the grain quality of winter wheat is reduced (Dupot et al., 2006; Haberle et al., 2008). To increase the nitrogen content in the grain, the leaf fertilizer application is used, usually in the period between end of stem elongation and beginning of flowering. The ability of leaves to uptake foliar applied urea in late development phases and to utilize the uptake nitrogen
for the formation of grain was studied in our experiments. It was found that in the period of plant transition to the generative phase the uptake of foliar applied urea is rapid and effective (Table 5). Under favourable weather almost 90% of the applied amount was found in plants. The nitrogen taken up at start of heading was at first translocated to the last internodium as a temporary storage and then to the ear. During harvest 95% of acquired nitrogen was stored in grain (Fig. 5). It shows a high efficiency of utilization of nitrogen acquired in the period of grain formation. Also Gooding et al., (2007) found that the nitrogen taken up from the foliarly applied urea at beginning of flowering or after anthesis was very quickly transported to the grain, 35 days after the application up to 67% from the absorbed N was found in the grain. The authors also observed that the utilization of N from urea depended among other on the year, grown variety and fungicide application.

Tab. 5 Effect of fungicide Tango Super on the uptake of foliar applied urea by wheat leaves during reproductive phase of development. Average ± SD; P=0,05; red colour – statistisical valuation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urea (Acquaired % $^{15}$N from applied CO($^{15}$NH$_2$)$_2$)</th>
<th>Urea + Tango (Acquaired % $^{15}$N from applied CO($^{15}$NH$_2$)$_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h (P=0,0037; F=36,98; Df=5)</td>
<td>19,2±3,8 a</td>
<td>48,4±5,6 b</td>
</tr>
<tr>
<td>4 days (P=0,0036; F=37,64; Df=5)</td>
<td>53,1±3,0 a</td>
<td>74,1±3,8 b</td>
</tr>
<tr>
<td>11 days (P=0,1804; F=2,63; Df=5)</td>
<td>84,5±2,1</td>
<td>78,5±4,8</td>
</tr>
<tr>
<td>Harvest (P=0,2179; F=2,36; Df=5)</td>
<td>90,6±4,4</td>
<td>87,1±1,3</td>
</tr>
</tbody>
</table>
Fig. 5  Translocation of N derived from foliar applied urea into growing parts of wheat plant. Urea was applied alone or together with fungicide Tango Super Average ± SD.

Simultaneous application of fertilizers and plant protection products is used mainly to decrease cost and because of expected synergic effects (Blandino and Reyneri, 2009). In our experiment it was found that with the simultaneous application especially the initial rate of uptake of foliar applied urea is influenced which may increase the uptake efficiency even under less favourable weather conditions in the period after application. Simultaneously, no effect on subsequent translocation and thus utilization for grain formation was observed.
4. Conclusions

The aim of this thesis was the evaluation of physiological and agronomic aspects of uptake and utilization of main forms of N in wheat.

Wheat belongs to the species sensitive to toxic effect of ammonium ion such as to the species with negative reaction to low pH too. In plants with NH\textsubscript{4}\textsuperscript{+} as the only source of nitrogen toxicity symptoms – limited root growth, lower R/S ratio, deficiency of essential cations (K\textsuperscript{+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+}) or accumulation of ammonium ions in the oldest leaves – were observed. These symptoms were largely suppressed by increasing pH of cultivation solution. Low pH also had negative effects on plants from the nitrate treatment. The toxic effect of ammonium ions is decreased by the higher availability of both forms of nitrogen. It seems that a strong acidification of rhizosphere caused by the ammonium ion uptake is the main reason of negative growing reaction of wheat plants.

Kinetic parameters of uptake (V\textsubscript{max} and K\textsubscript{m}) characterizing high-affinity uptake system, can be used for assessment of intra and inter species differences in effectiveness of nitrate and ammonium uptake. In both studied species of wheat (T.aestivum and T.durum) a higher capacity (V\textsubscript{max}) for the uptake of ammonium ions when compared to nitrates, but simultaneously a better affinity for nitrate (lower K\textsubscript{m}) was found. In all studied cultivars development of root system as a reaction to the form of supplied nitrogen was more or less influenced. There was a higher R/S ratio in plants growing under NO\textsubscript{3}\textsuperscript{-} than in NH\textsubscript{4}\textsuperscript{+}-fed plants. The capacity for uptake of both ions was substantially modified by size of root system. Intra and inter species comparison shown significant differences in the ability to nitrate uptake; in case of ammonium ions the differences were less distinctive. Evaluation of intra- and inter-species differences of uptake efficiency from the outer environment by means the determination of kinetic parameters of uptake systems could be used as a marker for selection of breeding material.

The rate of uptake of both nitrogen forms (NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+}) is influenced by the current state of the plant and is substantially
regulated by the rate of their assimilation and translocation to shoot. The necessity of fast ammonium assimilation in roots lead subsequently to substantial inhibition of its uptake. Assimilated nitrogen was then translocated to young growing leaves. More slowly taken up nitrate were rapidly translocated to the mature leaf where they were assimilated so that no substantial limitation of their uptake occurred.

If both ions are available in medium in the same concentration, uptake of ammonium ion is higher than nitrate. The rate of nitrate uptake in wheat was negatively influenced by the presence of ammonium ions – their uptake was decreased to one fourth compared to their uptake from nitrate medium only. The uptake of ammonium wasn´t influenced at all. Plants taking up nitrogen from the mixture of both ions thus acquired aproximately by 20% of nitrogen more than plants growing only on one of N forms.

The rate of uptake of all nitrogen forms is linearly dependent on temperature. In temperature ranges 2 °C – 20 °C, the rate of ammonium uptake is highest among N sources. In higher temperature, regulation of their uptake by insufficient rate of assimilation and thus their accumulation in roots was observed. The rate of nitrate uptake is two-time smaller than the rate of ammonium uptake. The maximum rate of nitrate uptake is already at 15 °C Urea uptake was the slowest from all forms. With respect to its rapid degradation in soil, importance of its uptake by plants is only slight.

Penetration of various forms of nitrogen through the cuticle and subsequent uptake into epidermis and mesophyll cells depends on their chemical nature. Ammonium (cation form) was the most easily taken up form by wheat leaves. The ammonium uptake into the cells of leaves was not so significantly limited by their assimilation rate, and that is why the ammonium nitrogen was the best-utilized form of nitrogen. The nitrate uptake (the anion form) was slower and moreover the high amounts of nitrate were accumulated in the exposed leaf, probably as a storage form in vacuoles. Urea (non-polar molecule) probably penetrated easily through the cuticle, but its uptake into cells was such as in roots, slower. Urea, like
ammonium ions, was metabolized rapidly and subsequently was used as a nitrogen source in growing tissues.

**Wheat leaves are able to uptake foliarly applied urea even during the generative phase of its development and urea can be subsequently fully utilized during the grain formation process.** Under favourable conditions plants acquire almost 90% of the applied amount at start of heading and 95% of this amount was found in the harvested grain. Foliar application of urea together with plant protection products (Tango Super) positively influenced the initial rate of its uptake which could improve the efficiency of utilization of the applied fertilizer under unfavourable environmental conditions.
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