Fixed nitrogen in agriculture and its role in agrocenoses

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Received: February 23rd, 2021; Accepted: May 12th, 2021; Published: May 19th, 2021

Abstract. On typical low-humus black soils in short crop rotations with legumes (25–33%) and without them, it was found that depending on the set of crops in crop rotation and application of fertilizer rates, nitrogen yield per crop is from 355 kg ha⁻¹ to 682 kg ha⁻¹. The recommended fertilization system provided nitrogen compensation for crop yields by only 31–76%. Hence, in the plant-fertilizer system nitrogen deficiency varies from 161 to 370 kg ha⁻¹. The greatest nitrogen deficiency in the soil is observed in crop rotation without the use of fertilizers with the following crop rotation: peas-winter wheat-grain maize-spring barley. The main source of nitrogen for plants is soil nitrogen. In crop rotations with legumes, biological nitrogen is supplied from the air, which in quantitative terms per rotation in crop rotations with peas is 109–288 kg ha⁻¹, with soybeans 264–312, and with alfalfa 486 kg ha⁻¹. Biological nitrogen in crop rotations with peas and soybeans is reimbursed from 25 to 62%, in crop rotation without legumes - 9% (non-symbiotic nitrogen fixation), and in crop rotation with alfalfa - 89% of the total nitrogen removal with the crop.

Key words: biological nitrogen, legumes, nitrogen balance, nitrogen fixation, short crop rotations.
INTRODUCTION

The level of soil fertility and crop yield largely depend on the reserves and mobility of nitrogen in the soil. Insufficient application of fertilizers and the absence of legumes reduce the content of organic matter and nitrogen in the soil. The current state of nitrogen balance in the soils of Ukraine does not correspond to its optimal parameters. Alfalfa, sainfoin and clover are the most powerful assimilation of biological nitrogen. The nitrogen fixation coefficient of these crops is 0.7–0.8. In general, perennial legumes attract from 100 to 300 kg ha$^{-1}$ of nitrogen to the biological cycle.

Nitrogen is a biogenic element of the Earth planet, and the main component of living matter, which plays a crucial role in the lives of plants and animals. A powerful nitrogen reservoir is the Earth's atmosphere, where its reserves are about 4 trillion tons. There is on average about 80 thousand tons of molecular nitrogen above each hectare of land in the air, which is a source of replenishment of bound nitrogen in the soil (Patyka et al., 2015; Toom et al., 2019; Karpenko et al., 2020; Tonkha et al., 2021).

The productivity of terrestrial and aquatic ecosystems, as well as the biosphere in general, depends on the sources of nitrogen. Providing green plants with nitrogen is a rather difficult task, as higher plants are not able to use free nitrogen in the air as a source of nitrogen nutrition. The studies have shown that all living organisms, including plants, constantly need available forms of nitrogen and have no ways to reserve it. They are in the ‘ocean’ of molecular nitrogen. Of all biodiversity of living nature, only a small number of organisms are able to supply themselves with nitrogen, while microorganisms-nitrogen fixers provide not only themselves but also the entire biosphere with biological nitrogen, as well as its reservation in the form of various nitrogen compounds (Eckert et al., 2001; Aras Mohammed Khudhur & Kasim Abass Askar, 2013; Manabu Itakura et al., 2013; Frans de Bruij, 2015; Steenhoudt & Vanderleyden, 2000; Hryhoriv et al., 2020; Novák et al., 2020).

The only way to create nitrogen reserves is to convert it into a specific soil organic matter - humus. Only soils have the ability to accumulate bound nitrogen during biological fixation and play the role of the only long-term bank of this element. In terms of significance for wildlife, the phenomenon of nitrogen fixation carried out by microorganisms in symbiosis with plants is compared with another global process of the Earth planet, namely photosynthesis (Zakharchenko et al., 1974; Kudeyarov, 1989; Patyka et al., 2015; Karpenko et al., 2020a).

The scientific researches have shown that under the conditions of field crop with insufficient application of fertilizers and the absence of legumes there is a gradual decrease in the content of organic matter and nitrogen in the soil. This is due not only to mechanical tillage and intensification of oxidative processes, but also the alienation of nitrogen with crop yields. Thus, the cost of humus (mineralization) for the cultivation of cereals reaches 0.5–0.7 and even 1.0 t ha$^{-1}$, including 25–50 kg ha$^{-1}$ of nitrogen. Under row crops and especially in conditions of pure vapors, the mineralization of organic matter and the lack of nitrogen in the soil is further enhanced (1.5–2.0 t ha$^{-1}$, including 75–100 kg ha$^{-1}$ of nitrogen), which subsequently leads to general deterioration of physical, physicochemical properties and nutrient status of the soil (Zakharchenko & Shilina, 1968; Zakharchenko et al., 1974; Karpenko et al., 2019; Telekalo & Melnyk, 2020).
Due to comprehensive and in-depth studies of the cycle and balance of plant nutrients in agriculture of Ukraine, the parameters of the cycle are set, the complex multifaceted transformation of nitrogen in the system ‘soil-fertilizer-plant-water’ is studied, and the optimal levels of return of nutrient elements to the soil with fertilizers in crop rotation of different climatic zones of Ukraine are determined. This, in turn, largely defined the high level of crop productivity and farming (Pirozhenko, 1963; Zakharchenko & Medvid, 1972; Shilina et al., 1983; Tsvei et al., 2008; Litvinov et al., 2020; Karbivska et al., 2020). If it is permissible on black soils for some time to increase yields due to the use of soil nitrogen reserves, then on poor soils of Polissia it is necessary to create a deficit–free and even excess nitrogen balance.

Hence, there is an urgent need to address the problem of restoring and preserving soil fertility, obtaining environmentally friendly agricultural products of high quality. The growing interest of scientists in the biological conversion of molecular nitrogen into biological one under the action of diazotrophic microorganisms in symbiosis with plants in natural ecosystems and agroecosystems is quite clear (Aras Mohammed Khudhur and Kasim Abass Askar, 2013; Manabu Itakura et al., 2013; Steenhoudt & Vanderleyden, 2000).

The most powerful assimilate of biological nitrogen in crop production is perennial legumes - clover, sainfoin, alfalfa - whose nitrogen fixation coefficient is 0.7–0.8. During the growing season, these crops accumulate from 30 to 38 kg of biological nitrogen for each ton of products in the dry matter. In general, perennial legumes attract from 100 to 300 kg ha\(^{-1}\) of nitrogen to the biological cycle, of which 75–200 kg is a pure product for the plant; the rest of the nitrogen is consumed by the plants from the soil. The assimilation capacity of legumes is slightly lower than perennial legume grasses. The nitrogen fixation coefficient is 0.4–0.5, and the rest of the nitrogen they use from the soil. For each ton of dry matter of the main and by-products lupine and fodder beans assimilate 20–27 kg from the air; peas, soybeans and others - 10–15 kg of nitrogen. Much of the biological nitrogen (sometimes up to 90%) during the growing season is concentrated in the aboveground mass, usually alienated from the field, and the rest remains in the soil in the roots and post–harvest residues, causing nitrogen gain in the soil after mineralization (Hamkalo, 2004; Kots & Patyka, 2009; Patyka et al., 2001; Patyka et al., 2003; Kots, 2011; Patyka, 2014; Yermolaev et al., 2014; Tanchyk et al., 2017; Karbivska et al., 2019).

Due to the ability of legumes to enter into symbiosis with specific for a particular species or group of species nodule bacteria, they can absorb up to 125–350 kg ha\(^{-1}\) of nitrogen during the growing season in the soil and climatic conditions of Ukraine (Zakharchenko & Shilina, 1968; Shilina et al., 1983; Pirozhenko,1993; Patyka et al., 2015; Litvinov et al., 2019). Due to symbiotic nitrogen fixation, legumes form high yields of cheap vegetable protein without the use of expensive, energy-intensive and environmentally hazardous mineral nitrogen fertilizers. After harvesting, more than 30% of biologically fixed nitrogen remains in post-harvest and root residues and is used by subsequent crops.

The goal of the work is to reveal the parameters of biological nitrogen fixation depending on the saturation of short-term crop rotations with the legume component.
MATERIALS AND METHODS

Field studies were conducted in a stationary field experiment during 2004–2011 in the subzone of unstable humidification of the Forest–Steppe of Ukraine at the Panfil Research Station of the National Scientific Centre ‘Institute of Agriculture of National Academy of Agrarian Sciences’ (Panfily village, Yahotyn district, Kyiv region) (Table 1). The experiment started in 2001, included 16 variants, with a four-time replication. The cultivation area was 90 m², the tested area under crops was 60 m². Cattle’s dung with litter was used for grain maize and sunflowers 10 t ha⁻¹ if calculate for 1 hectare of crop rotation fields, the other crops used an afteraction.

Table 1. Scheme of stationary experiment on the study of short crop rotations

<table>
<thead>
<tr>
<th>Variant of rotation</th>
<th>Alternation and fertilization of crops in crop rotation</th>
<th>Average crop rotation rate of fertilizers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Organic, Mineral, t manure</td>
</tr>
<tr>
<td>1</td>
<td>peas, winter wheat, grain maize, spring barley</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>peas, winter wheat, grain maize, spring barley</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>peas, winter wheat, grain maize, spring barley</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>perennial legumes, winter wheat, barley + grasses</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>peas, winter wheat, sunflower, spring barley, grain maize</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>soybean, winter wheat, grain maize, spring barley</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>buckwheat, winter wheat</td>
<td>–</td>
</tr>
</tbody>
</table>

The soil cover of the experimental area is typical low-humus coarse-grained light loamy black soil. The humus content in the arable layer (0–20 cm) varies from 3.08 to 3.15 % (by the Tyurin method). The soil is characterized by a high content of phosphorus - 233–270 mg kg⁻¹, high and medium content of exchangeable potassium (80–100 mg kg⁻¹ of soil) by the Chirikov method. The reaction of the soil solution is weakly acidic - pH_KCl - 5.7 by potentiometry, the degree of saturation of the absorption complex with bases is high (85–99%). The agronomic value of organic fertilizers (cattle’s dung without litter) according to the main food compounds in 1 ton of dung is: N - 4.0 kg, P₂O₅ - 2.0 kg, K₂O - 4.0 kg.
According to the observations of Yahotyn Meteorological Station (Yahotyn), the average annual air temperature is 7.3 °C; the average long-term precipitation is 442 mm and it varies from 250 to 670 mm; the relative humidity is 78%; the average duration of the growing season is 202 days.

Meteorological conditions during the years of research were quite contrasting, as evidenced by significant deviations from the long-term average in terms of precipitation and average monthly air temperature. Analysis of heat and moisture supply by the humidification coefficient (Hc) by Ivanov (Ivanov, 1946) shows that in all years of research the driest conditions were in April and May. This fact affected the vegetation of the studied crops to some extent (Fig. 1).

Notes: Humidification coefficient - the ratio of annual precipitation to evaporation for the same period 
Hc = P/f, where P is the amount of precipitation (mm), and f is the evaporation for the same period (%). 
Evaporation calculation according to Ivanov:  
$f = 0.018 \times (t + 25) \times (100 - R)$, where t – is the average temperature for the period (°C/year), R – is the average relative humidity (%).

Figure 1. Characteristics of heat and moisture supply of the stationary experiment during the growing season in the years of research on the humidification coefficient by N.M. Ivanov.

In general, the most favorable for the formation of crop productivity were 2004–2006, 2008 and 2009 and 2011. Arid conditions of crop vegetation developed in 2010, which was characterized by the most significant and extreme deviation of air temperature during the growing season. Based on the quantitative indicators of individual components of crop rotation in the soil-fertilizer-plant system obtained in the experiment, its full balance in the system of short crop rotations for 2004–2011 was compiled. The calculations are based on the method of comparing (ratio) of its expenditure and compensation items (Zakharchenko et al., 1974, Shilina et al., 1983). When compiling the balance, we did not consider the input of nitrogen with atmospheric precipitation and carried out by infiltration waters from the root layer of the soil, since
these sources of ‘input’ and ‘consumption’ of nitrogen in terms of parameters, as studies have shown, do not play a significant role in the balance. Therefore, in addition to accounting for its amount removed from the field by crops, the item ‘costs’ includes losses of nitrogen from fertilizers in gaseous form, and the item of income includes the amount of nitrogen in mineral and organic fertilizers and crop seeds. The input of biological nitrogen into the crop rotation system due to symbiotic and non-symbiotic nitrogen fixation was determined by the difference between the final indicators of nitrogen balance in the plant-fertilizer system and indicators of quantitative changes in the total nitrogen content in the soil during rotation.

RESULTS AND DISCUSSION

Biological nitrogen fixation is a powerful factor in the system of preservation and reproduction of soil fertility, increasing the productivity of agrocenoses. According to the results of the studies in short crop rotations with legumes (25–33%) and without them, it was found that depending on the set of crops in crop rotation and NPK application in the norm of 136–142 kg ha\(^{-1}\) of crop rotation area with legumes, crop nitrogen removal by rotation was from 355 kg ha\(^{-1}\) in the control variant (without fertilizers) up to 443–682 kg ha\(^{-1}\) when applying fertilizers (Fig. 2). This fertilizer system provided reimbursement of nitrogen costs for crop production by only 31–76%. Thus, a significant nitrogen deficit is created in the plant-fertilizer system, the value of which varied from 161 to 370 kg ha\(^{-1}\).

![Graph showing nitrogen balance in the plant-fertilizer system](image)

**Figure 2.** Nitrogen balance in the plant-fertilizer system, average for 2004–2011.

Note. Crop rotation variant: 1, 2, 3 – peas-winter wheat-grain maize-spring barley; 4 – alfalfa-wheat winter-grain maize-spring barley; 5 – peas-winter wheat–sunflower–spring barley–grain maize; 6 – soybean-winter wheat-grain maize; 7 – buckwheat-winter wheat.

Fertilizer rate per 1 ha of crop rotation area: 1. without fertilizers; 2. N\(_{45}\)P\(_{42}\)K\(_{55}\); 3. manure, 10 t + N\(_{45}\)P\(_{42}\)K\(_{55}\); 4. N\(_{45}\)P\(_{42}\)K\(_{55}\); 5. manure, 10 t + N\(_{40}\)P\(_{46}\)K\(_{62}\); 6. manure, 10 t + N\(_{40}\)P\(_{43}\)K\(_{53}\); 7. N\(_{45}\)P\(_{50}\)K\(_{50}\).
With such a significant nitrogen deficiency in the plant-fertilizer system, a decrease in the total nitrogen content in the soil was observed in the control crop rotation: pea-winter wheat-grain maize-barley (without fertilizers), where the main source of nitrogen for plants was soil nitrogen. In a similar crop rotation, under the mineral fertilization system (N<sub>60</sub>P<sub>42</sub>K<sub>55</sub> per 1 ha of crop rotation area) only 32% of nitrogen removal was compensated by fertilizers. This pattern was observed in two-field crop rotation: buckwheat–winter wheat, without legumes. The absolute values of nitrogen consumption in the soil in these crop rotations were much lower than its deficit in the plant-fertilizer system, which indicates an additional supply of nitrogen to the soil and plants from natural sources, namely due to biological nitrogen (symbiotic and non-symbiotic nitrogen fixation).

The studies have shown that in the soil–plant-fertilizer system (Table 2), in all variants of crop rotations due to the receipt of biological nitrogen, fixed from the air by legumes and partially existing in the soil microorganisms - nitrogen fixers, the result was positive, which in quantitative relation to crop rotations with peas was 109–288 kg ha<sup>-1</sup>, with soybeans - 312, and in crop rotations with alfalfa - 468 kg ha<sup>-1</sup>.

**Table 2. Biological nitrogen, average for 2004–2011**

<table>
<thead>
<tr>
<th>Fertilizer system (rate of fertilizers per 1 ha of crop rotation area)</th>
<th>Biological nitrogen (symbiotic and non-symbiotic nitrogen fixation) total</th>
<th>Including by fixing legumes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg ha&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>% from removal of crops</td>
</tr>
<tr>
<td>Peas-winter wheat-grain maize-spring barley control (without fertilizers)</td>
<td>+109</td>
<td>28</td>
</tr>
<tr>
<td>N&lt;sub&gt;45&lt;/sub&gt;P&lt;sub&gt;42&lt;/sub&gt;K&lt;sub&gt;55&lt;/sub&gt;</td>
<td>+261</td>
<td>48</td>
</tr>
<tr>
<td>manure, 10 t + N&lt;sub&gt;45&lt;/sub&gt;P&lt;sub&gt;42&lt;/sub&gt;K&lt;sub&gt;55&lt;/sub&gt;</td>
<td>+294</td>
<td>48</td>
</tr>
<tr>
<td>alfalfa-winter wheat-grain maize-spring barley</td>
<td>N&lt;sub&gt;45&lt;/sub&gt;P&lt;sub&gt;42&lt;/sub&gt;K&lt;sub&gt;55&lt;/sub&gt;</td>
<td>+468</td>
</tr>
<tr>
<td>peas—winter wheat—sunflower—spring barley-grain maize manure, 10 t + N&lt;sub&gt;46&lt;/sub&gt;P&lt;sub&gt;46&lt;/sub&gt;K&lt;sub&gt;62&lt;/sub&gt;</td>
<td>+288</td>
<td>40</td>
</tr>
<tr>
<td>soybean—winter wheat-grain maize manure, 10 t + N&lt;sub&gt;40&lt;/sub&gt;P&lt;sub&gt;43&lt;/sub&gt;K&lt;sub&gt;53&lt;/sub&gt;</td>
<td>+312</td>
<td>62</td>
</tr>
<tr>
<td>buckwheat—winter wheat</td>
<td>N&lt;sub&gt;45&lt;/sub&gt;P&lt;sub&gt;50&lt;/sub&gt;K&lt;sub&gt;50&lt;/sub&gt;</td>
<td>+21*</td>
</tr>
</tbody>
</table>

Note. * – due to non-symbiotic nitrogen fixation.

Due to biological nitrogen in crop rotations with peas and soybeans it was reimbursed from 28% to 62%, in crop rotation without legumes - 9% (non-symbiotic nitrogen fixation), and in crop rotation with alfalfa - 89% of the total nitrogen removal with the crop. Nitrogen increase due to fixation from the atmosphere by legumes was the highest in crop rotation with alfalfa - 107 kg ha<sup>-1</sup> per year and soybeans - 94 kg year<sup>-1</sup>; lower increase was in crop rotation with peas - from 17.3 to 63.5 kg ha<sup>-1</sup> per year. Nitrogen fixed by legumes prevailed in the total increase of biological nitrogen in crop rotations. Thus, biological nitrogen in crop rotations (20–50%, and in crop rotations with perennial legumes up to 90%) is able to compensate for nitrogen costs and thus...
significantly reduce the need for mineral nitrogen. Thus, nitrogen fixation in crop rotation largely depends on the role and saturation of the crop rotation with legumes.

The current state of nitrogen balance in agriculture in Ukraine does not correspond to its optimal parameters. According to the State Statistics Committee of Ukraine, the current level of fertilizer application in the fields of the country is: organic fertilizers - 0.5 t ha\(^{-1}\), mineral fertilizers - 80 kg ha\(^{-1}\), of which mineral nitrogen is 67%. The share of the area on which fertilizers are applied is for organic ones - 1.2%, for mineral fertilizers - 39.8% of the total area of agricultural land (State Statistics Committee of Ukraine. http://www.ukrstat.gov.ua.).

Scientifically substantiated level of compensation for nitrogen removal from all sources of income, on average in Ukraine, according to the results of long experimental studies and calculations based on them should be at least 110% (in Polissia 130, Forest-Steppe - 100–110, Steppe - 100%) (Patyka, 2014; Shilina et al., 1983). This means that with high crop productivity, adequate nitrogen removal of 100 kg ha\(^{-1}\) to create its positive balance, which will ensure the reproduction of soil fertility, for each hectare of arable land you need to return at least 110 kg of nitrogen Potential opportunities for the supply of biological nitrogen due to symbiotic nitrogen fixation are: perennial legumes - 30–35 kg per 1 ton of dry matter of the main product; legumes (peas, soybeans, beans) - 10–15 kg per 1 ton of dry matter of the main and by-products; lupine, fodder beans - 20–27 kg per 1 ton of dry matter of the main and by-products (Zakharchenko et al., 1952; Zakharchenko & Shilina, 1968; Zakharchenko et al., 1974).

Calculations of biological nitrogen supply showed that about 10% of legumes in the structure of sown areas (including 2.83% of perennial legumes) and the average yield of legumes - 1.80–2.64 t ha\(^{-1}\), perennial legumes - 4.01 t ha\(^{-1}\) due to symbiotic nitrogen fixation for each hectare of arable land in 2018 we received additionally only 6.08 kg of nitrogen (Table 3).

That is, the total supply of biological nitrogen due to symbiotic nitrogen fixation in the country's agriculture is determined solely by the area under legumes and in particular perennial legumes in the structure of sown areas.

CONCLUSIONS

Effective in the system of short-rotation crop rotations on chernozem is nitrogen replenishment due to symbiotic and associative nitrogen fixation. Against the background of the use of fertilizers at high crop yields, the intake of biological nitrogen due to nitrogen fixation in crop rotations with peas is 261–294 kg ha\(^{-1}\), soybeans - 312 kg ha\(^{-1}\), perennial grasses (alfalfa) - 468 kg ha\(^{-1}\).

By introducing a legume component into the crop rotation, it makes it possible to compensate, due to nitrogen fixation, from 40 to 89% of the nitrogen consumption from fertilizers and soil for the growing crops.

The priority measure for the effective use of biological nitrogen of legumes in agriculture should be a scientifically grounded structure of sown areas with the optimal share of these crops at the national level and at the level of each farm, regardless of its land ownership form.

<table>
<thead>
<tr>
<th>Legume crop</th>
<th>Sowing area</th>
<th>Crop yield of main products, t ha⁻¹</th>
<th>Yield of dry matter of main product, cha⁻¹</th>
<th>Yield of dry matter of by-products (in dry matter), c ha⁻¹</th>
<th>The ratio of the main and by-products (in dry matter), c</th>
<th>Yield of dry matter of by-products, t ha⁻¹</th>
<th>Total dry matter yield of main and by-products, t ha⁻¹</th>
<th>Gross collection of dry matter of main and by-products, t ha⁻¹</th>
<th>Fixation of nitrogen from the air kg per 1 ton of dry matter of main and by-products</th>
<th>Total kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain legumes (peas, beans, vetch etc.)</td>
<td>568.4</td>
<td>1.75</td>
<td>1.80</td>
<td>1.55</td>
<td>1:1.3</td>
<td>2.02</td>
<td>3.57</td>
<td>2,029.188</td>
<td>10</td>
<td>20,291.880</td>
</tr>
<tr>
<td>Soybeans</td>
<td>1,709.0</td>
<td>5.25</td>
<td>2.64</td>
<td>2.28</td>
<td>1:1.6</td>
<td>2.65</td>
<td>4.93</td>
<td>8,425.370</td>
<td>10</td>
<td>84,253.700</td>
</tr>
<tr>
<td>Perennial legumes (clover, alfalfa, sainfoin)</td>
<td>920.6</td>
<td>2.83</td>
<td>4.01 (hay)</td>
<td>3.37</td>
<td>–</td>
<td>–</td>
<td>3.37</td>
<td>3,102.422</td>
<td>30</td>
<td>93,072.660</td>
</tr>
<tr>
<td>Total of legumes</td>
<td>3,198.0</td>
<td>9.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>197,618.240</td>
</tr>
<tr>
<td>Arable land of 2018</td>
<td>32,500.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>On 1 ha of arable land</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.08</td>
</tr>
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REFERENCES


