Fertilizer for the Treatment of Iron Chlorosis
Physico-chemical and agro-chemical properties

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Iron chlorosis represents a physiological phenomenon with negative impact on the quality and quantity of grape production in the vineyards. The bicarbonates are considered to be the main group of compounds which immobilize the iron in the soil in forms not assimilable by the plants. The grape production in conditions of iron deficiency can be improved significantly by applying foliar fertilizers. The best result in mitigating the effects of iron deficiency were obtained by the application of the fertilizers which, in addition to a complex mineral composition, also have a content of organic matter in the form of humic substances or protein hydrolysate.

Keywords: fertilizers, chlorosis, grapevine, humic

The treatment and causes of iron chlorosis in vineyards has been the subject of several researches since the great Phylloxera blight until now. In time knowledge was acquired about the soil and the availability of nutrients in it, about the nutrition and physiology of the grapevine and especially about the manner of absorption, translocation and utilization of iron in the soil-plant system.

In Romania, the species most affected by iron chlorosis are the grapevine and the peach tree; it can occur less frequently in other species, such as beans, broccoli, cauliflower, fruit-bearing shrubs etc. [1-6]. Iron chlorosis occurs mainly on calcareous soils, especially because of certain factors such as: high humidity and deficient aeration [7-11] leading to an increased concentration of bicarbonate ions, which are at a great extent responsible for the immobilization of the iron [12-21]. Iron plays a complex role in plant nutrition, its main functions being related to its role in the formation of hemoproteins (catalase, cytochromoxydase, hemoglobin etc.) and in proteins with S- and Fe- bonds.

The lack of iron disrupts the entire physiological activity of the plant and its manifestation can be seen in the entire cycle culture. The symptoms of iron chlorosis can be seen on the entire plant, as follows: the leaves remain small, the veins are still green but the interveinal space turns yellow, and in more severe cases the leaf edges become necrotic and eventually the leaf falls, the yellowing (chlorosis) starts from the growing tips of the plant because of the very low mobility of the iron, the shoots are thin, debilitated, with short zigzagged internodes, the grapes remain small, degenerated, undevolved [23, 31-38].

Several methods have been adopted for the prevention and control of iron chlorosis but currently it is believed that the second most efficient method for keeping the plants in a proper physiological condition after choosing a resilient rootstock is the foliar fertilization. Initially the measures consisted in the administration of iron salts (iron sulphate, ferric sulphate, ferrous ammonium phosphates and sulfates etc.) in the soil and on the leaves, but the efficiency was rather low. Then foliar fertilization started to prove its efficiency especially when iron was complexed with EDTA, DTPA and EDDHA [30-39].

The iron fertilizers used in viticulture (and not only in viticulture) belong to two large categories, namely inorganic and organic. In a synthesis of iron sources, [3, 6, 16, 19, 40-43] mention the following substances:
- ferrous sulfate FeSO₄ hydrated with four or seven molecules of water, with a 20 – 23% content of iron.
- ferric sulfate Fe₂(SO₄)₃ hydrated with 20% iron.
- ferrous ammonium phosphate Fe(NH₄)PO₄ hydrated with 42% iron.
- iron phosphate Fe₃(PO₄)₂ octohydrate (also known as vivianite), with over 32% iron.
- ferrous ammonium phosphate Fe(NH₄)₂PO₄ monohydrate, with 29% iron.

The use of inorganic iron fertilizers had good results when applied on the soil only where the soil was lacking this element. One of the main problems was and will remain the fact that a calcareous soil can immobilize larger quantities of iron than the ones that can be administered by means of a normal technology, so the efficiency of the iron salts is reduced when applied on the soil. However, there are recommendations [6], regarding the administration of ferric sulfate along with manure in the hole where the grapevine is planted, since it is known that an environment rich in organic substances prevents iron from being blocked in inaccessible forms.

Foliar application had better results, 25 – 100 times more efficient than the application on the soil. Iron immobilization in the epidermis of the leaves takes place at a lower rate than in the soil. However, because of the low mobility of iron in the plant, the treatments must be repeated as new leaves grow. Complexation of iron (and also of other micronutrients) with citric acid, resulting in iron citrate, is also used in the fertilization practice. Unfortunately, the disadvantage of iron citrate is its being photolabile.

The iron bound in organic combinations, the so-called iron chelates, came to be used on a larger scale due to certain stability advantages they have. From among the chelates, the following ones are worth being mentioned [16, 39-41, 44, 45]:

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- Fe-EDTA (the iron salt of the ethylenediamine-tetraacetic acid, with a 6 - 12% iron content, has a good chemical stability but a pH below 7;  
- Fe-DTPA (the iron salt of the diethylenetriaminepentaacetic acid), with a 10% iron content, has a good chemical stability even at a pH above 7;  
- Fe-EDDHA (the iron salt of the ethylenediamine-N,N’,N”-tris(2-hydroxyphenylacetic acid), with a 6% iron content, has a high chemical stability, up to pH 10;  
- Fe-HEDTA (the iron salt of the hydroxyethyl-ethylenediaminetriacetic acid).  

The chelates are generally fertilizers with a much higher efficiency than those with inorganic iron, mainly due to their good chemical stability on a wide pH range, especially in the alkaline zone, where the inorganic iron rapidly turns into inaccessible forms.

The main disadvantage of the chelates is their high price, for which reason their application in the soil is limited to high value cultures, such as the vegetables or flowers in protected spaces. That is why in the general agricultural practice the foliar application is the most widely used and it proved to be successful in the alleviation of the iron chlorosis symptoms when applied in quantities of 100 - 200 mL of chelated iron per hectare. In such conditions it can be stated that the foliar application is the most efficient method of fertilization with iron.

There are numerous data on the effectiveness of fertilizers containing organic matter from different sources [46-53] including the use of protein hydrolysates [54-64,74,76,79] and humic substances [65-70,75] in agriculture that have seen remarkable growth due to proven positive effects.

This work aims at obtaining certain fertilizers with iron for the treatment and prevention of iron chlorosis and the physicochemical and agrochemical characterization thereof.

We evaluated the influence of four foliar fertilizers in the process of treatment and control of iron chlorosis, namely: one with Fe complexed with DTPA, one complex with iron, microelements and macroelements and two complex organomineral fertilizers with humic acids and protein hydrolysate.

**Experimental part**

**Obtaining experimental fertilizers**

The fertilizers used in the experiments were prepared in the laboratory, by specific techniques, at National Research and Development Institute for Soil Science Agrochemistry and Environment - RISSA Bucharest. Four fertilizers with different physical and chemical characteristics were used, one of them, procured from the free market, being used as control. The four variants of foliar products are as follows:

- V2 - is a foliar fertilizer based on iron complexed with diethylenetriaminepentaacetic acid (DTPA), in a 6% concentration, control fertilizer;  
- V3 - foliar fertilizer prepared at National Research and Development Institute for Soil Science Agrochemistry and Environment - RISSA, with macro, mezzo and microelements, having a 19 g/L Fe content;  
- V4 - foliar fertilizer prepared at National Research and Development Institute for Soil Science Agrochemistry and Environment - RISSA, with macro, mezzo and microelements, having a 23 g/L Fe content and humic acids in the form of potassium humate;  
- V5 - foliar fertilizer prepared at National Research and Development Institute for Soil Science Agrochemistry and Environment - RISSA, with macro, mezzo and microelements, with a 26 g/L Fe content and collagen hydrolysate.

The procedure of preparing the fertilizers included several stages: first, preparation of a solution of primary and secondary potassium phosphates, then preparation of an NPK-type solution by adding nitrogen, preparation of a solution of chelated microelements (Fe, Zn, Mg, Mn, Cu, Mo, B, S), preparation of a solution of humic acids or protein hydrolysate, then mixing the solutions. In the end the products were decanted and filtered. The scheme of preparation of the experimental fertilizers is presented in figure 1 [71-73].

![Fig. 1. Scheme for preparing experimental fertilizers](http://www.revistadechimie.ro)

The solution of primary and secondary potassium phosphates was prepared by neutralization of concentrated phosphoric acid (85%) using technical potassium carbonate (98%); from this reaction a mixture of mono and di-potassium phosphate resulted, at the ratio of $K_2(HPO_4)\cdot KH_2PO_4 = 0.6 - 0.75$. The reaction took place under continuous stirring and at a constant temperature of 25-30°C.

The NPK-type solution was prepared by adding a nitrogen source in multiple forms, nitric, amidic and ammonia, to the solution prepared as described above.

The solution of microelements was prepared by dissolving of sources (sulfates or chelates) of Fe, Zn, Mg, Cu, Mn, boric acid. The content of organic substances in the fertilizers was in the range of 190 - 260 g/L, in which 10 g/L were humic substances or protein hydrolysate.

The potassium humate was prepared by extraction from lignite with a 1.5% KCO$_3$ solution, pH = 11.5, at the temperature of 75°C, under stirring for 6 h. The humic acids thus extracted were characterized by the thermal gravimetric analysis and by the FT-IR spectrum (Fourier transform infrared spectroscopy).

The (collagen) protein hydrolysate was obtained from bovine hide, following the neuter hydrolysis for 8 h at the temperature of 120°C. The amino acid composition of the collagen hydrolysate used in preparing the fertilizer is presented in table 1 [80].

- Fe-EDTA (the iron salt of the ethylenediamine-tetraacetic acid, with a 6 - 12% iron content, has a good chemical stability but a pH below 7;  
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![Fig. 1. Scheme for preparing experimental fertilizers](http://www.revistadechimie.ro)
The three fertilizer variants obtained experimentally were characterized from the physicochemical point of view at the Laboratory for Fertilizer Testing and Quality Control within National Research and Development Institute for Soil Science Agrochemistry and Environment – RISSA, a laboratory accredited by RENAR, in accordance with the testing methods specified in Regulation (EC) No. 2003/2003 of the European Parliament and of the Council relating to fertilizers.

Establishing the conditions for testing

The agrochemical experiments were conducted in vegetation pots, in order to show the influence of the four foliar fertilizers with different characteristics, as compared to a non-treated control, on the grapevine under nutritive stress.

The certified plants used in the experiment were produced at the Bujoru Center of Research & Development in Viticulture and Vinification, located in Targu Bujor, Galai District. The grapevine is of the Muscat Ottonel variety, grafted on the rootstock Berlandieri x Riparia Selection Oppenheim 4 Clone 4, in the viticultural practice named SO4-4.

The soil used in planting the grapevine was analyzed in the National Research and Development Institute for Soil Science Agrochemistry and Environment – RISSA laboratory [77] and its properties are presented in table 2.

The determinations were carried out in the laboratory on the soil sample: pH potentiometrically in aqueous suspension, using a combined glass-calomel electrode; flame photometry, atomic absorption spectrometry methods; the organic carbon content was determined by the Walkley-Black method modified by Gogoași; the total nitrogen content by the Kjeldahl method; the mobile forms of phosphorus and potassium, soluble in ammonium acetate lactate solution (AL) at pH 3.7, after Egnér-Rhiem-Domingo, were determined by spectrophotometry, respectively flame photometry.

The grapevines planted as described above were drip irrigated under a pressure of 1.5 bar, using a main water supply pipe made of HDPE (high density polyethylene) PN9 with the diameter of 25 mm and the connections were made of LDPE (low density polyethylene). Each row of grapevine had a 17 mm LDPE watering line on which there were 4-outlet drippers, each dripper having a 4 L/h flow. Each outlet ending in a "dripping pen" was placed at the root of a grapevine. For the optimal control of the watering, the route was equipped with a programmer and a solenoid. A rain sensor was also installed, to stop the watering when the weather was rainy. Pending on the season, the environmental temperature and the phenophase, the water quantity ranged between 1 liter/week/vine and 1 liter/day/vine, less at the beginning of the vegetation period, more in summer time, and a gradual decrease in autumn.

During the vegetation period, in order to prevent and treat the diseases and pests, five phytosanitary treatments were applied in the following periods: burgeoning – sprouting, before flowering, after flowering, growth of grapes and mellowing and before harvesting.

In order to induce the nutritive stress, no radicular fertilization was applied to the grapevines. In order to induce iron chlorosis, in spring, 150 g ammonium bicarbonate was applied to each vine, in 3 portions of 50 g each, at 1 week intervals. The targeted effect was double: one the one hand to block the iron in the forms of bicarbonates and other compounds which are formed at a high pH and on the other hand to induce a growth leap by means of the ammonium, in order to disrupt the balance between the vegetative growth and the radicular absorption of the iron.

The plants so treated started to show the typical signs of iron chlorosis, first by slowing down of their growth, then by the slight yellowing of the tip of the leaves, which is the sign of iron deficiency.

The foliar fertilization started from the 3rd quarter of the month of May. The treatments were applied by spraying the fertilizer solutions on both sides of the leaves. Each

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**Table 1**

<table>
<thead>
<tr>
<th>No.</th>
<th>Amino acid</th>
<th>Average concentration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glycine</td>
<td>33.03</td>
</tr>
<tr>
<td>2</td>
<td>Proline</td>
<td>12.51</td>
</tr>
<tr>
<td>3</td>
<td>Alanine</td>
<td>12.03</td>
</tr>
<tr>
<td>4</td>
<td>Glutamic acid</td>
<td>8.20</td>
</tr>
<tr>
<td>5</td>
<td>Hydroxyproline</td>
<td>7.67</td>
</tr>
<tr>
<td>6</td>
<td>Asparagine</td>
<td>5.10</td>
</tr>
<tr>
<td>7</td>
<td>Arginine</td>
<td>4.54</td>
</tr>
<tr>
<td>8</td>
<td>Serine</td>
<td>3.48</td>
</tr>
<tr>
<td>9</td>
<td>Lysine</td>
<td>2.75</td>
</tr>
<tr>
<td>10</td>
<td>Valine</td>
<td>2.32</td>
</tr>
<tr>
<td>11</td>
<td>Leucine</td>
<td>2.31</td>
</tr>
<tr>
<td>12</td>
<td>Phenylalanine</td>
<td>1.80</td>
</tr>
<tr>
<td>13</td>
<td>Threonine</td>
<td>1.56</td>
</tr>
<tr>
<td>14</td>
<td>Isoleucine</td>
<td>1.05</td>
</tr>
<tr>
<td>15</td>
<td>Histidine</td>
<td>0.97</td>
</tr>
<tr>
<td>16</td>
<td>Taurine</td>
<td>0.38</td>
</tr>
<tr>
<td>17</td>
<td>Methionine</td>
<td>0.28</td>
</tr>
</tbody>
</table>

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**Table 2**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter determined</th>
<th>Method of determination</th>
<th>Value obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total nitrogen</td>
<td>Kjeldahl</td>
<td>0.28%</td>
</tr>
<tr>
<td>2</td>
<td>Organic carbon</td>
<td>Wet oxidation</td>
<td>2.54%</td>
</tr>
<tr>
<td>3</td>
<td>C/N ratio</td>
<td>Calculus</td>
<td>9.07</td>
</tr>
<tr>
<td>4</td>
<td>Pnj mobile phosphorus</td>
<td>Photoclinorimetric</td>
<td>9.12 ppm</td>
</tr>
<tr>
<td>5</td>
<td>Kj mobile potassium</td>
<td>Flame photometer</td>
<td>133.33 ppm</td>
</tr>
<tr>
<td>6</td>
<td>pH</td>
<td>Electrometric</td>
<td>5.44</td>
</tr>
<tr>
<td>7</td>
<td>Fe extraction in EDTA</td>
<td>Atomic absorption</td>
<td>123.18 ppm</td>
</tr>
<tr>
<td>8</td>
<td>Fe extraction in DTPA</td>
<td>Atomic absorption</td>
<td>138.52 ppm</td>
</tr>
<tr>
<td>9</td>
<td>Total Fe (mineralization in acids)</td>
<td>Atomic absorption</td>
<td>2.30%</td>
</tr>
</tbody>
</table>
vine was treated with 50 mL of fertilizing solution having the concentration of 1% fertilizer, to which 0.05 mL of Vital 90 (adjuvant to foliar treatment) was added. Four foliar treatments were performed, one in May, two in June and one at the beginning of July. Before each foliar treatment, the chlorophyll concentration index (CCI) was measured and 7 days after the last foliar fertilization, the CCI was read again in order to include in the experiment the effect of the last fertilization. The treatments were applied in the morning, when the weather was calm, wind free, and the temperature was below 30°C.

The equipment makes it possible to quickly and non-destructively assess, in situ, the chlorophyll content in leaves. Leaf chlorophyll content provides valuable information about physiological status of plants. CCM-200 calculates the chlorophyll content index (CCI), which is defined as the ratio of percentage of transmission 935 nm to 635 nm through leaf tissues [78].

After the completion of the stages of fertilization and reading of the CCI, the grapevines continued to receive the general treatment as described above.

At the end of September the grapes were harvested, weighed, and the must obtained from then was analyzed in order to determine the total content of sugars and the total acidity.

The experiment aimed at assessing the influence of four foliar fertilizers on the plants. The experiment comprised 5 experimental variants, i.e. 4 fertilizers (V2 – V5) and a control sprayed with water (V1), with 3 repetitions for each variant, in randomized selection and marked in accordance with the variant and the repetition.

Statistical analysis
The experimental design was a randomized design with three replications. Data from experiments were statistically computed for statistical parameters and variance analysis.

Results and discussions
Characterization of experimental fertilizers
FTIR analysis
Characterization of humic acids added to the fertilizer named V4 was carried out by FTIR spectroscopy. For this purpose, the equipment used has VERTEX 70 FT-IR spectrometer equipped with ATR diamond monoreflexion cell (PIKE Miracle®). FTIR analysis was carried out in the wavenumber range of 4000 – 600 cm⁻¹, with detector at 4 cm⁻¹ resolution and 32 scans per sample and background correction for CO₂/H₂O.

Fourier transform infrared (FTIR) spectra provides valuable information on the functional properties of HAs. The FTIR spectrum exhibits the typical major peaks for natural HAs and relevant spectral regions were assigned according to the previously reported assignments [81-8]. The FTIR spectrum of humic acids (fig. 2) showed the peak at about 2934 cm⁻¹ that represents aliphatic C–H stretching in C–H2 and C–H3. The IR absorption band at 1614 cm⁻¹ can be due to C–O stretching of carboxyl, or the C–N stretching of amide I. There is also a strong band at approximately 1708 cm⁻¹, which is attributed to C–O stretching vibration of carboxylic acid and the intense band at 1217 cm⁻¹ indicates the presence of C–O and OH groups of carboxyl acids.

![Fig.2. FTIR spectra of humic acids](image)

Testing of experimental fertilizers
The agrochemical experiment performed on grapevines in vegetation pots by the extraradicular use of fertilizers with iron and organic substances in an NPK-type matrix with secondary and microelements generated the results presented below.

Figure 3 shows the differentiated evolution of the grapevines in the 5 experimental variants regarding the chlorophyll concentration index (CCI). The trend of this

<table>
<thead>
<tr>
<th>Variants of fertilizers obtained in laboratory</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (Nt)</td>
<td>84.81</td>
<td>43.19</td>
<td>59.08</td>
</tr>
<tr>
<td>Phosphorus (P₂O₅)</td>
<td>25.80</td>
<td>16.59</td>
<td>22.60</td>
</tr>
<tr>
<td>Potassium (K₂O)</td>
<td>29.40</td>
<td>24.00</td>
<td>27.00</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.09</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.89</td>
<td>0.46</td>
<td>0.45</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>19.71</td>
<td>23.22</td>
<td>26.57</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.14</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>4.61</td>
<td>0.86</td>
<td>2.44</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>0.91</td>
<td>0.81</td>
<td>0.47</td>
</tr>
<tr>
<td>Sulfur (S)</td>
<td>20.07</td>
<td>18.33</td>
<td>18.33</td>
</tr>
<tr>
<td>Humic substances / hydrolysate</td>
<td>-</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3: CHEMICAL COMPOSITION DETERMINED FOR THE FERTILIZERS USED
Table 4

PHYSICAL PROPERTIES DETERMINED FOR THE FERTILIZERS USED

<table>
<thead>
<tr>
<th>No.</th>
<th>Physical properties for the fertilizing solution</th>
<th>Measure unit</th>
<th>Values determined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH, conc. 5%</td>
<td>pH unit</td>
<td>V3</td>
</tr>
<tr>
<td>2</td>
<td>pH, conc. 1%</td>
<td>pH unit</td>
<td>5.71</td>
</tr>
<tr>
<td>3</td>
<td>pH, conc. 0.5%</td>
<td>pH unit</td>
<td>6.31</td>
</tr>
<tr>
<td>4</td>
<td>pH, conc. 0.25%</td>
<td>pH unit</td>
<td>6.84</td>
</tr>
<tr>
<td>5</td>
<td>Conductivity, 1% solution</td>
<td>microS/cm</td>
<td>2450</td>
</tr>
<tr>
<td>6</td>
<td>Conductivity, 0.5% solution</td>
<td>microS/cm</td>
<td>1364</td>
</tr>
<tr>
<td>7</td>
<td>Conductivity, 0.25% solution</td>
<td>microS/cm</td>
<td>726</td>
</tr>
<tr>
<td>8</td>
<td>Density of fertilizing solution</td>
<td>g/ml</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Fig. 3. Evolution in time of chlorophyll concentration index (CCI)

Fig. 4. Average grapes crop and its statistical indicators (g/vine)

Fig. 5. Average mass of grape (g)
parameter was an increase of the index in all the variants except for the control. The greatest increase was in variants 4 and 5, where the fertilizers contained, in addition to the micro and macro elements, also organic substances either in the form of humic acids, or in the form of protein hydrolysate. There followed variants 2 and 3, namely the iron fertilizer and the complex fertilizer with micro and macro elements but without organic substances.

From a quantitative point of view, in all the experimental variants, except for the non-treated control, the average crop per vine and the average mass of the grape increased (figures 4 and 5). The highest influence was seen in variants V4 and V5, namely the organo-mineral fertilizers, followed by variants V2 and V3, namely the iron fertilizer and the complex mineral fertilizer with micro and macro elements.

Following the application of the fertilizers, the values of the chlorophyll concentration index increased in all of the experimental variants (figure 6), but the highest values were recorded following the application of the organo-mineral fertilizers V4 and V5.

Conclusions

From the experiment regarding the extraradicular application of iron fertilizers to grapevine, performed in vegetation pots, bicarbonate ion, by the nutritive stress had a positive effect as far as the targeted parameters are concerned, namely the chlorophyll concentration index, the average mass of the grape, the contents of sugars and the acidity.

The weakest influence, at times with values below the theoretical limit of significance, was recorded in variant V2, the commercial product based on Fe chelate; these results indicate that the addition of iron improves the metabolism of the plant and the chlorophyll content, but this is insufficient for the rehabilitation of the plant.

Variant V3, the complex mineral fertilizer with micro, mezzo and macroelements, had a better and statistically more significant influence, even though its iron content is only about one third as compared to variant V2; this fact shows that the curative effect of the fertilizer is stronger when the iron is accompanied by the other nutrients.

The strongest and most significant influence was seen with the two complex fertilizers with microelements, macro elements and organic substances, the variants V4 and V5. As compared to V3, the improving action of the organic substances, both of the humic substances and of the protein hydrolysate is obvious.

Fig. 6. The values of the chlorophyll concentration index

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