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PRELIMINARY PHOTOSYNTHESIS EXAMINATIONS OF THERMOFIL EVERGREEN ORNAMENTAL SHRUBS IN HUNGARY

MELEGIGÉNYES ÖRÖKZÖLD DÍSZCSERJÉK ELŐZETES FOTOSZINTÉZIS VIZSGÁLATA MAGYARORSZÁGON

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Abstract

The purpose of this research was to determine the climatic- ecological demand of thermofil broadleaf evergreen ornamental shrubs. On three different habitats: in field conditions, in container and on hillside was investigated the fluorescence induction experiments with PAM-2000 chlorophyll-fluorimetry, which was used to measure the photosynthesis 2 quantum yield of the plant.

Keywords: PAM-2000, thermofil evergreen shrubs

Absztrakt

Melegigényes lomblevelű örökzöld díszcserjék magyarországi klimatikus-ökológiai igényét szeretnénk megismerni. Három különböző élőhelyen: szántóföldön, konténerben és domboldalon végeztünk fluoreszcencia indukciós kísérleteket egy PAM-2000 nevű klorofill-fluoriméterrel, amellyel a II. fotokémiai rendszer aktuális kvantumhatékonyságát mértük.

Kulcsszavak: PAM:2000, melegigényes örökzöld cserjék

Detailed abstract

According to the climate change scenarios the climate in Hungary (USDA Hardiness Zone 5-7) will be dryer, richer in sunshine and winters are expected to become warmer because of the Mediterranean effect. As the nursery gardeners have to prepare themselves for the climate change therefore at the Pannonia University thermofil (USDA Hardiness Zone 7-9) broadleaf evergreen ornamental shrubs were examined for their adaptational capacity to the continental conditions.

Because of geohistorical- and climate conditions the flora of our country is poor in broadleaf evergreens therefore our aim was to enrich the offering of nurseries with this barely known taxons.

The species participating in the experiment living on three different habitats since 2007 were as follows:

Aucuba japonica ‚Rozzanie'*, Cotoneaster franchettii, Elaeagnus pungens* ‚Maculata Aurea', *Ilex cornuta, Ligustrum sinense, Ligustrum texanum, Nandina domestica, Osmanthus heterophyllus, Phillyrea angustifolia, Photinia x fraseri* ‚Red Robin'*, Prunus lusitanica, Sarcococca hoockeriana, Viburnum cinnamonifolium, Viburnum tinus.*

Chlorophyll fluorescence measurements have been widely used in plant physiology and especially in ecophysiology. In situ pulse amplitude modulated (PAM-2000) fluorometry was used to investigate the photosynthesis of the above-mentioned species on three different habitats: in field conditions, in container and on hillside. In case of significant interactions among factor, monofactorial effects were analysed separately. To test the relationship between the PSII quantum yield and the relative water content of the leaves was used regression analysis.

On the basis of our examinations was determined that *Ilex cornuta* liked the container, *Prunus lusitanica* the field conditions, *Elaeagnus pungens* 'Maculata Aurea' the hill-side, while *Cotoneaster franchettii* lived well in all three habitats.

According to the results were getting closer to get acquainted with the ecological demands of these species and varieties.

Az éghajlatváltozást modellező forgatókönyvek szerint Magyarország (USDA télállósági zóna 5-7) éghajlata szárazabbá, napsütésben gazdagabbá válik és a telek várhatóan melegebbek lesznek a mediterrán hatás miatt. Mivel a faiskolásoknak is el kell kezdeniük a felkészülést a klímaváltozásra, ezért a Pannon Egyetemen melegigényes (USDA télállósági zóna 7-9) lomblevelű örökzöld díszcserjék kontinentális feltételekhez való adaptációs képességének vizsgálatába kezdtünk.

Mivel a földtörténeti- és éghajlati feltételek miatt hazánk flórája szegény a lomblevelű örökzöldekben, ezért célunk az volt, hogy a faiskolák kínálatát gazdagíthassuk ezekkel a hazánkban alig ismert taxonokkal.

A faj a kísérletben részt vevő élő három különböző élőhelyeken 2007 óta a következők voltak: *Aucuba japonica* ‚Rozzanie'*, Cotoneaster franchettii, Elaeagnus pungens* ‚Maculata Aurea', *Ilex cornuta*, *Ligustrum sinense, Ligustrum texanum, Nandina domestica, Osmanthus heterophyllus, Phillyrea angustifolia, Photinia x fraseri* ‚Red Robin'*, Prunus lusitanica, Sarcococca hoockeriana, Viburnum cinnamonifolium, Viburnum tinus.*

Klorofill fluoreszcencia méréseket már széles körben használják a növényélettannal foglalkozók. Az általunk használt impulzus amplitúdó modulált (PAM-2000) fluoriméterrel megmértük a fent említett fajok aktuális fotoszintézis intenzitását három különböző élőhelyeken: szántóföldi körülmények között, a konténerben és domboldalon.

Mivel a különböző tényezők között jelentős volt a kölcsönhatás, ezért az egyes faktorok hatását külön is elemeztük. A PSII aktuális kvantumhatásfokának és a levelek relatív víztartalmának kapcsolatának a leírására regresszióanalízist használtunk.

A statisztikai vizsgálat igazolták azt, amit szemrevételezés során is tapasztaltunk, miszerint az *I. cornuta* számára a konténer, a *P. lusitanica* számára a szántóföldi körülmények, az *E. pungens* ‚Maculata Aurea' számára pedig a dolomit alapkőzeten lévő barna erdőtalaj az ideális a fejlődéshez, míg a *C. franchettii* fejlődése számára mindhárom élőhely kedvező.

Mindez hozzásegít minket ahhoz, hogy ezen fajok ill. fajták klimatikus-ökológiai igényét megismerjük hazánk éghajlatán is és tanácsokat tudjunk adni a felhasználásukat illetően.

Abbreviations

ΔF - Increase of fluorescence yield, Fm' - Fs, induced by a Saturation Pulse;
ΦPSII= Y: The actual quantum yield of PS II photochemistry in the light-adapted state;
df – degree of freedom
DW - dry weight of the leaf;
F – fluorescence intensity at any point;
Fm – maximum fluorescence yield in dark-adapted state;
Fm' – maximum fluorescence yield in light-adapted state;
Fo – minimum fluorescence yield in dark-adapted state;
Fo' – minimum fluorescence yield in light-adapted state;
Fs – fluorescence in steady-state;
Fv/Fm – quantum yield of PS II photochemistry in the dark adapted state (equivalent to [*Fm-Fo*]/*Fm*);
Fv=Fm- Fo, variable fluorescence in dark adapted state ;
FW - fresh weight of the leaf;
LSD 5% - Least Significant Difference;
MS – Mean Square;
PS II – Photosystem II;
p-value - Measure of how possible the sample results are, assuming a true null hypothesis. A smaller p-value indicates less likely sample results;
R-square – gives the percent of variance due between group variation ;
RWC - Relative water content of the leaf;
SS – Sum of Squares;
Y =ΔF/Fm' = (Fm' − Fs) /Fm', yield, photochemical efficiency of PSII in light adapted state

INTRODUCTION

Because of geohistorical- and climate conditions the flora of our country is poor in broadleaf evergreens, but it is very important not to forget, that the broadleaf evergreen shrubs are plants of generally slightly warmer and milder winter areas [16].

Our aim was because of the climate-change to enrich the offering of nurseries with these in Hungary barely known taxons, therefore these shrubs has been studied in tree habitats to determine the ecological demands of these species/varieties.

The climate conditions and the expected change of climate in Hungary

Over the last 150 years the surface air temperature of the Earth has risen by 0.6–0.8°C, with certain deviations from one region to another [19]. The climate of Hungary is jointly determined by oceanic, Mediterranean and continental effects, and these, together with the relief features of the Carpathian Basin, lead to very variable weather conditions [5].

The analysis of the temperature data of Keszthely

In the data series of annual mean temperatures the warming up is significant (0.49ºC/100 years) between 1901 and 2000 [7]. The series of data extended until 2006 shows an even more intensive warming (0.58ºC/100 years) [8].

The analysis of the precipitation data series of Keszthely

The distribution of the data indicates that in the examined period that was the majority of the years had a lower amount of precipitation. Both the series of the running averages and the climate normals show that the amount of precipitation decreased in the second half of the 20th century. During the examination of the number of periods without precipitation can be concluded that farmers have to face at least one 15-day or two 10-14-day periods without precipitation in each growing season. As a conclusion can be established that the decrease of precipitation can be regarded as one of the consequences of global climate change [8].

About photosynthesis and fluorescence

Photosynthetic apparatus of plants is sensitive to stress conditions caused by anthropogenetic and natural stress factors [14, 15]. Photosystem II (PSII) is a sensitive component of the photosynthetic apparatus which may be temporarily affected by environmental stress before any irreversible structural damage is observable [18]. Subsequent changes in chlorophyll *a* fluorescence emission, arising mainly from the PSII, can then be observed: chlorophyll fluorescence provides information on almost all aspects of photosynthesis and the response of plants to environmental factors such as stress and nutrient availability [2, 11, 9, 12, 10]. Chlorophyll fluorescence works on the principle that photosynthesis is one of the core functions in the physiology of plants. The functional state of photosynthesis has been considered an ideal physiological activity to monitor the health and vitality of plants [3]. Assessing the health or integrity of the internal apparatus driving the photosynthetic process within a leaf using chlorophyll fluorescence provides a rapid and accurate technique of detecting and quantifying plant tolerance to stress.

The simplicity and rapidity of the ‘‘saturating pulse’’ method of in vivo Chl fluorescence measurements, combined with the commercial availability of truly portable and user-friendly instruments, made this technique very popular among plant physiologists and ecophysiologists [6]. Pulse-amplitude-modulation chlorophyll fluorescence [17] has been shown to be a suitable technique for studying the tolerance of the photosynthetic apparatus to the environmental constraints, as it allows a non-invasive assessment of PSII function and the allocation of absorbed energy into photochemical and non-photochemical processes.

Stress factors decrease the efficiency of photosynthesis and suppress the variable fluorescence correspondingly. The ratio Fv/Fm also decreases with the increasing effect of stress factors. Changes of Fv/Fm values actually reflect the physiological status of trees. Fv/Fm determination is powerful to evaluate the physiological state of plant in urban ecosystems.

Chlorophyll *a* fluorescence is now used widely as a non-invasive and rapid technique for estimating photosynthetic performance in plants. In recent years, the value of chlorophyll fluorescence as a technique in plant physiology has been greatly increased by the availability of suitable instrumentation and an increased understanding of the processes which regulate fluorescence yield. Perhaps the single most useful fluorescence parameter is ($\Delta F/Fm$’), which estimates the yield of PS II photochemistry (ΦPSII) and frequently shows a strong, quantitative relationship with the quantum yield of CO2-assimilation [4, 13].

MATERIALS AND METHODS

Plant material

During the experiment, the following 14 species or cultivars of evergreen ornamental shrubs were examined in collection plot of University Pannonia Georgikon Faculty since 2007: *Aucuba japonica* ‘Rozzanie’ (*Cornaceae*), *Cotoneaster franchettii* (*Rosaceae*), *Elaeagnus pungens* ‘Maculata Aurea’ (*Elaeagnaceae*), *Ilex cornuta* (*Aquifoliaceae*), *Ligustrum sinense* (*Oleaceae*), *Ligustrum texanum* (syn.: *Ligustrum japonicum* var. Texanum) (*Oleaceae*), *Nandina domestica* (*Berberidaceae*), *Osmanthus heterophyllus* (*Oleaceae*), *Phillyrea angustifolia* (*Oleaceae*), *Photinia x fraseri* ‘Red Robin’ (*Rosaceae*), *Prunus lusitanica* (*Rosaceae*), *Sarcococca hoockeriana* (*Buxaceae*), *Viburnum cinnamonifolium* (*Caprifoliaceae*), *Viburnum tinus* (*Caprifoliaceae*)

Experimental area

For the experiment was used 4400 m^2 area on three habitats, all belonging to the experimental farms of the University of Pannonia.

Habitat 1. 1750 m^2 is located on the Experimental Station for Fruit Cultures of the University, Keszthely. The field condition of the plot is plane and medium compacted brown forest soil. The groundwater level is below 200cm of the surface. The height of tilth is above 150cm. The top-soil is shallow. The soil is acidic-neutral, its physiological hardness as the humus content is low. The soil analysis data indicate a weakly good phosphorus and potassium supply. The supply of magnesium is good, of manganese and copper is abundant while the supply of zinc is optimal. The soil is poor in mineral nitrogen. The species of the Experiment 1 were planted in 3 repetitions, and according to their viability the plant distance was 2,5 x 1,5 m, 2,5 x 1,0 m and 1,0 x 1,0 m. Following the planting the soil surface around the plants were covered with chopped bark to protect weeds. Here were planted 30 plants per species or cultivars.

Habitat 2. 150 m^2 place of containers, is located on the garden of the Department of Horticulture of the University, Keszthely. This plot was earlier a demonstration field for ornamental plants and medicinal plants. The plants were taken into 10-30 litre pots (containers), filled with Agro CS RSII Professional soilmixture which contained the best quality of white and black peat with clay, adjusted chemical reactions. It was largely enriched with all nutritive and microelement. The pH-value was stabilized by clay. 8-10 plants per species or cultivars were planted.

Habitat 3. 2500 m^2, is located on Cserszegtomaj, on the Experimental Station for Wine Cultures of the University. Earlier also research works has been made– naturalization of subtropical plants like citrus and orange - using ditch technology. On the hillside the groundwater level is below 200cm. The height of tilth is changeable and stony. The top-soil is shallow-medium. The field condition is brown forest soil on dolomite bedrock. The soil is weakly alkaline, its physiological hardness is medium-high, its humus content is low. The concentration of phosphorus and potassium in soil is very good-good. The supply of magnesium is good, of manganese, copper and zinc is abundant. The level of mineral nitrogen is medium.

In the habitat 3 the test plants (6-8 plants per species/cultivars) were planted in mixed spots creating a closed population density. Those species which need also some shadowing, were planted in the near of trees grown earlier naturally on the field.

These 14 species were planted in the first half of June 2007.

Experimental protocol

The experiment was performed between 15 and 20 September 2009.

Fluorescence measurements

The effective quantum yield in a light adapted state was measured in the different habitats on the upper surface of mature, current year leaves. Measurements were randomised according to plants and were taken between 1100 and 1400 hours on sunny days. The light adapted leaves were measured with a distance clip, so the leaves were fixed in such a way that the measuring fibre optics and clip did not shape onto the surface at the moment of the measurement.

Fluorescence parameters (see [20] for terminology) were measured on the adaxial surface of mature leaves, using a portable pulse-amplitude-modulated fluorometer (PAM-2000, H. Walz, Effeltrich, Germany). The steady-state value of fluorescence (F_s) was measured, and a pulse of high intensity white light was used to determine maximum fluorescence in the light-adapted state (F_m'). The photochemical efficiency of PSII in the light, was calculated according to [4]: $\Phi PSII = Y = \Delta F/F_m' = (F_m' - F_s) / F_m'$

Measurements were made in twenty replicates per in each population.

Relative water content

The tissue water content of the leaf portions was determined according to [1]. After determining the fresh weight (FW), the samples were dried to a constant weight at 65 °C and allowed to cool down for 3h before determining the dry weight (DW).Tissue water content was calculated using the formula (FW-DW)/FW)x100. Measurements were made in five replicates per species/cultivars in each habitat.

Statistical evaluation

Statistical analysis were performed using mainly ANOVA’s for comparisons of means. In case of significant interactions among factor, monofactorial effects were analysed separately. To test the relationship between the parameters was used regression analysis. MS Office Excel 2003 (Microsoft, Redmond, Washington) was used for the statistical analysis.

RESULTS AND DISCUSSION

The results of the actual quantum yield measurements in the three different habitats are found in the Table 2. First the Y was analysed. On the basis of the Two-Way ANOVA both the habitats and both the species/varieties had a significant role in which habitats preferred the observed species/varieties. Because a significant interaction between species and between the pilot-sites, One-way ANOVA was carried out, the habitats were compared with the species and the species/varieties were compared with the habitats.

Table 1. The results of Two-way ANOVA
A kéttényezős varianciaanalízis eredményei

TWO-WAY ANOVA ΔF/Fm'	SS	df	MS	F	p-value	R-square%
Species	2,2	13	0,168340	26,4	1,15E-54	17,7
Habitats	1,7	2	0,831497	7,4	6,25E-04	13,4
Interaction	2,9	26	0,111769	17,6	4,79E-63	23,5
Error	5,6	882	0,006368			
Total	12,4	923			Sum R-square%	54,6
TWO-WAY ANOVA RWC	**SS**	**df**	**MS**	**F**	**p-value**	**R-square%**
Species	8411,8	14	600,8	20,38	3,92E-30	47,9
Habitats	769,4	2	384,7	3,49	3,25E-02	4,4
Interaction	3084,6	28	110,2	3,74	3,89E-08	17,6
Error	5308,0	180	29,5			
Total	17573,8	224			Sum R-square%	69,8

The comparison of species/varieties showed that the *Cotoneaster franchettii* was (R^2 %=0.6) insensitive to the different soil and topographical ability, while *Viburnum cinnamonifolium* (R^2 %= 76,5), the *Ilex cornuta* (R^2 = 72%) and *Osmanthus heterophyllus* (R^2 %= 68,7) were sensitive to the different experimental sites. 5 species/varieties (*Cotoneaster franchettii*, *Ligustrum sinense*, *Ligustrum texanum*, *Phillyrea angustifolia*, *Sarcococca hoockeriana*) showed no significant difference between the good quality field conditions and container conditions; 5 species/ varieties (*Aucuba japonica* 'Rozzanie', *Cotoneaster franchettii*, *Elaeagnus pungens* 'Maculata Aurea', *Photinia x fraseri* 'Red Robin' *Prunus lusitanica*) showed no significant difference between the container and the dolomite bedrock of the brown forest soil, while 3 species/varieties (*Cotoneaster franchettii*, *Ligustrum sinense*, *Viburnum tinus*) did not show any significant difference between the field and hillside conditions. *Ligustrum sinense* (R^2 %= 11,5), *Ligustrum texanum* (R^2 %= 13,4), and *Aucuba japonica* 'Rozzanie' (R^2 %= 17) showed somewhat sensitive reaction to the soil and terrain conditions.

If the species/varieties were compared, one could see that the plants responded very differently to the nursing in containers (R^2 %= 73,9). The *Cotoneaster franchettii*, *Osmanthus heterophyllus* and *Ilex cornuta* were developing quite well in containers, while for *Viburnum tinus, Viburnum cinnamonifolium*, *Nandinia domestica* and *Photinia x fraser* 'Red Robin' were not beneficial the nursing in containers for a longer period. The hillside habitat was shared the species/varieties (R^2 %= 48.6). This region is also favorable for the *Cotoneaster franchetti*, like *Ligustrum sinense*, *Prunus lusitanica* and *Elaeagnus pungens* 'Aurea Maculata', while for *Viburnum cinnamonifolium*, *Nandina domestica*, and *Sarcococca hoockeriana* was not so favourable the hillside exposed to sunlight . A good-quality arable land did not share this extent the species (R^2 %= 30,4), was not too much variation between them, which was almost consistent with the empirical status of recording.

To compare the RWC Two-way ANOVA was performed, according this the biggest difference was between species/varieties (R^2 %= 47,9), while there was hardly any difference between the habitats (R^2 % = 4,4).

One-way ANOVA was used to compare the habitats, one could see that the plants responded very differently to the hillside (R^2 % = 85,5), so to the container (R^2 % = 77,9) and even the field conditions shared the taxons (R^2 % = 54).

The species/varieties were also compared with One-way ANOVA, where was found that only in four taxon's RWC were significant differences between the different habitats: *Ligustrum sinense* (R^2 %= 73,6), *Phillyrea angustifolia* (R^2 %= 67,9), *Viburnum cinnamonifolium* (R^2 %= 66,9), *Sarcococca hoockeriana* (R^2 %= 57,8). Whereas these trends were very different from the Y, therefore, regression analysis estimated the correlation between parameters. The present experiment could not observe any significant relationship between RWC and Y. The reason for this and other affecting factors will be examined in the future.

On the basis of our examinations *Cotoneaster franchettii* liked all three habitats, but *Viburnum cinnamonifolium, Ilex cornuta* and *Osmanthus heterophyllus* were sensible for the examined habitats. These three species preferred the container conditions according our present experiment. The following 5 species/varieties were less sensible for the different habitats: *Aucuba japonica* 'Rozzanie', *Elaeagnus pungens* 'Maculata Aurea', *Ligustrum sinense, Ligustrum texanum, Nandinia domestica* and *Prunus lusitanica.*

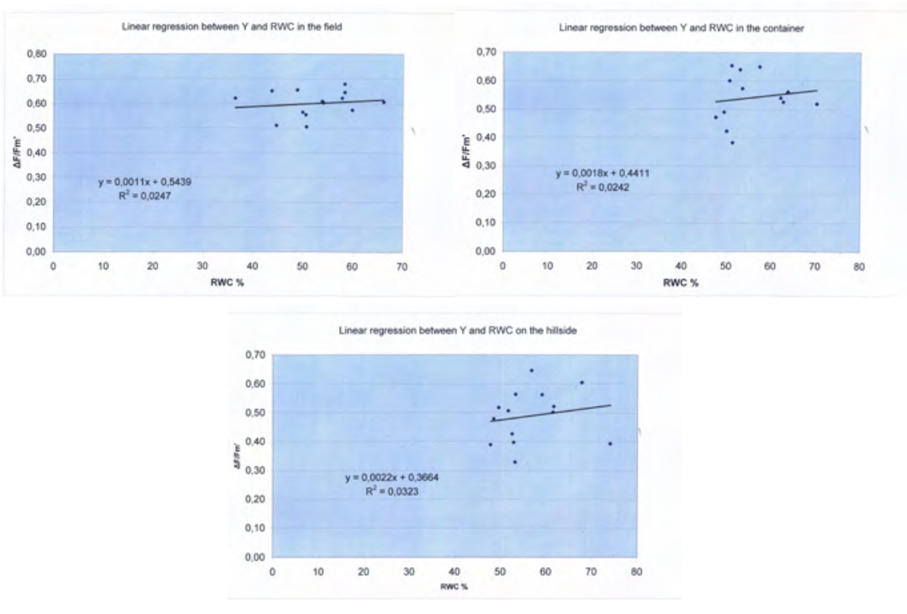

Figure 1-3. Linear regression between actual quantum yield and relative water content of leaves in the three different habitats
Lineáris regresszióanalízis a fotoszintézis aktuális kvantumhatásfoka és a levelek relatív víztartalma között a három különböző élőhelyen

Table 2. The photosynthesis activity of thermofil ornamental shrubs on three different habitats
Melegigényes díszcserjék fotoszintézis aktivitása három különböző termőhelyen

Plants	Quantum yield of phtosynthesis (ΔF/ F_m')						
	field condition	container	hillside	average	LSD5%	R-square%	p-value
A. japonica 'Rozzanie'	0,6066	0,5403	0,5212	0,5560	0,0499	17,0	**
C. franchettii	0,6506	0,6531	0,6459	0,6499	0,0247	0,6	0,836
E. pungens 'Maculata Aurea'	0,6222	0,5255	0,5619	0,5699	0,0427	24,9	***
I. cornuta	0,5647	0,6390	0,3964	0,5334	0,0401	72,0	****
L. sinense	0,5546	0,5192	0,6044	0,5594	0,0558	11,5	*
L. texanum	0,5739	0,5617	0,5016	0,5457	0,0484	13,4	**
N. domestica	0,5112	0,4711	0,3887	0,4570	0,0575	24,0	***
O. heterophyllus	0,6090	0,6490	0,4260	0,5613	0,0395	68,7	****
Ph. angustifolia	0,6214	0,6004	0,5175	0,5798	0,0370	35,8	****
Ph. x fraseri 'Red Robin'	0,6544	0,4897	0,5065	0,5502	0,0594	37,1	****
P. lusitanica	0,6789	0,5727	0,5632	0,6049	0,0337	48,0	****
S. hoockeriana	0,6030	0,5597	0,3929	0,5185	0,0606	46,0	****
V. cinnamonifolium	0,6457	0,4225	0,3285	0,4656	0,0467	76,5	****
V. tinus	0,5055	0,3824	0,4784	0,4555	0,0432	35,1	****
average	0,6001	0,5419	0,4881				
LSD5%	0,0456	0,0439	0,0539				
R-square%	30,4	73,9	48,6				
p-value	****	****	****				

Note: *:	p-value < 0,05
**:	p-value < 0,01
***:	p-value < 0,001
****:	p-value < 0,0001

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